PLANT PHYSIOLOGY

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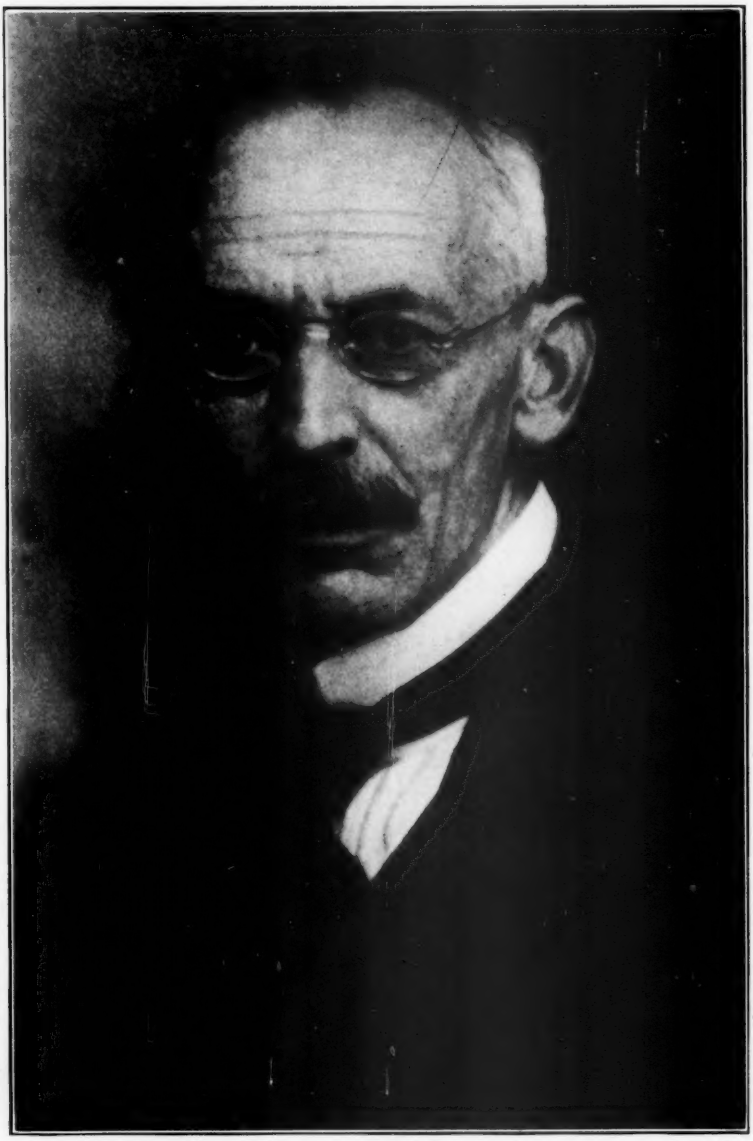
ERRATA

Page 22, table XI, column 4, for 5.06 read 5.08.
Page 23, second line below table XII, for 0.3271 read 0.2371.
Page 82, line 14 from bottom, for bulk read bulb.

Page 84, line 12 from bottom, for raising false stems read raising the false-stems.
Page 85, last line, for SCHWARTZ read SCHWARZ.
Page 92, second line of legend, fig. 13, for 12 read −2.
Page 93, average of −1, A, for 21.7 read 21.1.
Page 95, fig. 14, highest ordinate at left, for 130 read 135.
Page 101, citation no. 5, for SCHWARTZ read SCHWARZ.
Page 152, line 9 from bottom, for standard read saturated.
Page 223, line 17 from bottom, for (16) read 16.
Page 257, title of table IV, for WHITE read WITTE.
Page 258, line 6 from bottom, for are read is.
Page 264, line 3, for Hochaktiver read hochaktiver.
Page 372, line 2, for Linksstrasse read Linkstrasse.
Page 433, line 14, omit a before $Ca(NO_3)_2$.
Page 477, line 5 from bottom, for immerson read immersion.
Page 481, first line of footnote, for prophylene read propylene.



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MOVEMENT OF ORGANIC MATERIALS IN PLANTS

ALDEN S. CRAFTS

(WITH FOUR PLATES AND THREE FIGURES)

Introduction

Many theories have been proposed to explain the movement of foods in the plant but few of them seem sound when critically examined. Most of these theories fail to describe a mechanism capable of conducting the required amounts of material through the available channels with the necessary rapidity. Such a mechanism must exist and it must be compatible with the form and structure of the plant in which it serves.

The first theories on organic transport (**42, 27, 39**) were based upon ringing experiments but the interpretations of these experiments were so influenced by animal physiology as to render the theories untenable. HARTIG (**29**) first described phloem tissues and recognized their relation to food conduction. He proposed protoplasmic streaming (**30**) as an accelerating mechanism in the distribution of these materials and he also observed exudation from the phloem (**31**).

Following HARTIG a controversy as to which tissues functioned in conduction (**28, 49, 58, 32**) occupied the attention of investigators. NÄGELI (**49**) suggested changing turgidity as a cause for movement along sieve-tubes and DE VRIES (**17**) elaborated the protoplasmic streaming theory, supporting his hypothesis with some microscopical studies.

ZACHARIAS (**67**) and KRAUS (**41**) contributed data on the composition of the exudate from the cut phloem of cucurbits, their analyses indicating a high content of food materials. Little work has been done on phloem exudates since that time and the idea, developed by early botanists, that this material flowed from cut sieve-tubes (**25, 26**) has apparently never been contested. The anatomical studies of HILL (**33, 34**) and his predecessors (**65, 34**) and the physiological and morphological investigations of RUHLAND (**57**) and SCHMIDT (**60**) seem to justify a reasonable doubt as to

the function of the sieve-tube in conduction. If pores actually exist in the protoplasmic connections of these elements their extreme fineness makes questionable the importance of mass flow.

Ringing has survived as the principal method for studying translocation (**16, 37, 44**) though at best it can only hint as to the mechanism involved. The experiments of CZAPEK (**15**), CURTIS (**11, 12, 13**), and MASON and MASKELL (**46, 47, 55**) are typical of this method and they indicate quite conclusively that downward movement of organic materials takes place through the bark. The experiments of MASON and MASKELL show that concentration gradients exist in the phloem and that they are more or less related to the rate and direction of movement.

BIRCH-HIRSCHFELD (**4**) carried out a number of experiments attempting to demonstrate conduction of lithium and dyes through plant tissues. Finding movement through the phloem to be very slow she proposed that downward conduction occurs in the xylem. DIXON (**21**), and DIXON and BALL (**20, 22**) have emphasized the inadequacy of sieve-tubes as conducting elements and have further elaborated the mechanism proposed by BIRCH-HIRSCHFELD. Although their calculations in no way prove the impossibility of mass movement through the phloem, they show the necessity for mathematical analysis of the problem and suggest a method for measuring the fitness of any hypothesis.

CURTIS, finding that ringing interferes with the upward movement of nitrogen as well as with the downward movement of organic materials, advocates the protoplasmic streaming hypothesis as providing for movement in both directions through the same tissue at the same time. He gives no calculations as to the rates at which foods are moving but considers acceleration due to streaming to be rapid enough to account for growth and storage. In a late paper (**14**) he emphasizes the importance of living cells in this mechanism.

Other theories have appeared from time to time (**26, 43, 38**) but the one proposed by MÜNCH (**48**) is of the greatest significance in relation to the present work. He described a physical system composed of two osmometer cells containing solutions of different concentrations connected by a tube. When this apparatus was placed in pure water a mass flow took place resulting in a transfer of solute from the cell of higher concentration to the one of lower concentration. He applied this system to the plant assuming that photosynthesis maintains a concentration difference between assimilating cells and storage or meristematic tissues, the sieve-tubes acting as the connecting elements. He described experiments in which exudation from the cambium and excess growth resulted from interruption of the descending sap stream. Cutting the phloem caused a very rapid decrease

in exudation pressure, especially below the cut. This decrease in pressure was transmitted downward at a rate of ten centimeters per second for as far as six meters. MÜNCH calculated the rate of flow necessary to account for certain known increases in dry weight of trees, and estimated that about two and one half per cent. of the total water used by the tree circulates through the phloem system. He suggested that transfer of assimilate from leaf parenchyma to sieve-tubes takes place along the plasmodesma assuming that the property of selective permeability is confined to the outer layer of the protoplast.

Existing theories

The most prominent theories concerning food conduction in plants as described in the literature may be classified under two heads: (1) those favoring movement through the xylem and (2) those advocating the phloem as the channel.

(1) The first of these, as developed by DIXON and BALL, proposes that a solution of organic nutrients having passed from synthesizing cells of the leaf due to increased permeability will be drawn down through the xylem tubes by the water columns in tension and will be absorbed at lower levels by living cells and distributed laterally throughout the stem or root.

There are several serious objections to this mechanism based upon well known or easily demonstrated anatomical facts. In the first place the open junction through which the downward moving xylem sap flows to join the ascending transpiration current must be located at a considerable distance below the point of entry of the organic materials; in fact, for the nutrition of the root tip it must occur at the extreme lower end of the two tubes concerned. This is not the case in the normal plant. A series of experiments on the movement of dyes in leaves has shown that water movement may take place in any direction indicating that the xylem system forms an interconnected network of tubes in which movement is determined by pressure gradients which depend upon size of openings and rate of water loss. It seems therefore that if any particular portion of a leaf became permeable, allowing a flow of solution from the mesophyll cells into the xylem, it is improbable that this solution would move out of the leaf.

That a similar system, though not so closely connected, exists throughout the plant may be shown by cutting petioles and stems under eosin. The solution will flow backward until it meets a junction with another portion of the plant and will then join the ascending stream and finally arrive at a transpiring surface. The only way in which the underground portion of plants could be injected was by allowing the plants to absorb eosin until the transpiring surfaces were all killed, whereupon a complete reversal of flow set in, caused by a water deficit developed in those roots which were

surrounded by dry soil. In no case did the eosin solution move out into the root tips. In fact, in *Convolvulus arvensis*, upon which many of these experiments were made, it is impossible to explain the nutrition of the root tip on the basis of movement through the xylem because the radially arranged primary xylem strands remain isolated (**65**) from the secondary xylem by unlignified living parenchyma cells. In this case the descending stream of nutrient sap could not approach within several centimeters of the rapidly growing tips of main roots without crossing the barrier of living cells. It is difficult to see how it could do this if moved by tension resulting from transpiration. The roots of many woody plants are known to grow when the parts above ground are leafless and dormant. A mechanism dependent upon tension cannot explain nutrition of their growing points. A consideration of the structure of woody tissues lends further weight to this argument. It is a well known fact that lignified cell walls are permeable to water and dissolved substances though fairly resistant to rapid lateral movement (**65, 23**) so that any imbibition deficit (**61**) which existed in one portion of the xylem would be transmitted to the surrounding tissues. Then since pressure acts in all directions equally a pressure gradient in the longitudinal direction would also exist laterally, so that a solution flowing downward due to increased pressure in an individual vessel or chain of tracheids would also tend to spread to surrounding elements either through the wall or through pits (**2**). In tall trees, therefore, where the lowest leaves may be from 50 to 100 meters from root tips it seems highly improbable that longitudinal movement through the xylem could supply organic nutrients to the roots in view of the constant loss from the descending stream by lateral movement. The data presented by CURTIS, MASON and MASKELL and others make the xylem theory even more untenable; their experiments provide convincing evidence for the longitudinal transfer of carbohydrates through the bark.

(2) Of those theories which favor the phloem as the channel for transport, some consider the movement from cell to cell by a process similar to diffusion and possibly accelerated by protoplasmic streaming as the best explanation, others favor a mass flow through the sieve-tubes. The first of these theories is open to serious objection. The parenchyma and companion cells of the phloem are typical living cells with semipermeable protoplasts and are relatively impermeable to sugars. RUHLAND (**57**) found that he could plasmolyse these cells with hypertonic solutions of sucrose, fructose and glucose and that recovery was slight even after 24 hours. The same results were obtained with cucurbit tissues in the present work. Longitudinal sections of phloem tissue were allowed to absorb neutral red for a few minutes and then mounted in hypertonic sucrose solution. The cells were rapidly plasmolysed and showed no recovery after several hours.

These results indicate that if sugar were moving through the phloem the protoplasts of these cells would offer considerable resistance. It is doubtful if the acceleration due to protoplasmic streaming could account for the rates of movement which have been calculated as necessary. In the first place streaming has not been demonstrated to be universal in its occurrence in these tissues. BIRCH-HIRSCHFELD (**4**) found no evidence that it accelerated movement in her experiments, and BIERBERG (**3**) found acceleration to be only from 3 to 4 times that of normal diffusion. DIXON (**21**) calculated that a 10 per cent. sucrose solution would have to move 50 cm. per hour in order to account for the observed translocation into potato tubers. BIRCH-HIRSCHFELD (**4**) made similar calculations and MASON and LEWIN (**45**) calculated a rate of 88 cm. per hour for a 25 per cent. solution through the sieve-tubes of the greater yam. If this were moving through the parenchyma cells, the rate could be reduced to about 34 cm. per hour for a 25 per cent. solution or 88 cm. for a 10 per cent. solution. DE VRIES (**17**) observed a rate of streaming of from 0.2 to 0.4 mm. per minute in phloem cells. EWART (**24**) records rates up to 3 mm. per minute in water plants and in the present work a maximum rate of 2 mm. per minute was observed in phloem parenchyma cells of cucurbit stems. The maximum rate of 3 mm. per minute would be 18 cm. per hour which would not suffice even if the streaming cell constituted a perfect machine, and if the diffusion in and out through the cell wall and ectoplasm were neglected. CURTIS (**13**) sees little difficulty in this diffusion across end walls, terming them "occasional membranes"; but since these cells range from 0.5 mm. to 0.2 mm. or less in length the number traversed must necessarily be 20 or more per centimeter.

If the time required for recovery from plasmolysis in hypertonic sugar solutions is a satisfactory indication of the permeability of the protoplasmic layer for sugar molecules, then it must be granted that diffusion through the ectoplasm into the streaming inner layers at one end of the cell, and the subsequent movement out at the other end would be a very slow process. This hindrance, in addition to the time required for diffusion through the walls, would undoubtedly reduce the average rate to one incommensurate with that mentioned by DIXON. Finally, an estimate of the percentage of the total cross-sectional area of the phloem actually occupied at any one time by sugar moving in the proper direction, suggests that a concentration factor of serious magnitude is involved.

Considering next the possibility of a mass flow through the sieve-tubes we find that the narrowness of the pores through the sieve-plates constitutes a serious difficulty. This objection was apparently neglected by NÄGELI (**49**), SACHS (**58**), CZAPEK (**15**), and other early workers. EWART (**24**) calculates that half an atmosphere would be sufficient to produce a mass

movement of 5 mm. per minute through the 2000 sieve-plates, each having pores 2 μ in diameter and 10 μ in length, which he finds in 50 cm. of stem.

It is hard to harmonize this calculation with one which has been made in this work. It has long been known that cucurbit stems when cut will exude measurable quantities of a clear watery sap from the phloem tissues. The writer watched this exudation by means of a dissecting binocular microscope giving a magnification of 150 times and it is apparently confined to the phloem with the exception of a small amount arising from the chlorenchyma sheath which surrounds the stem near the outside. The xylem sap was under tension at the time that these observations were made so that the phloem exudate was drawn into the vessels until they became blocked by the jell which is formed upon contact of this exudate with the air.

In this experiment collections were made of the sap from 20 cut stems, the sap being removed to small test tubes by means of a pipette from the larger stems and by shaking directly into the tubes in the case of the smaller ones. Collections were made over periods of one and two minutes, the tubes corked and taken to the laboratory where the corks were removed and the weights determined, by difference, on an analytical balance. The tubes were then placed in an 80° oven equipped with a fan and thoroughly dried and reweighed to obtain dry weights. Immediately following collection portions of each stem were sectioned, projected on paper and the areas of phloem obtained at a given magnification. By higher magnification of phloem groups, areas of sieve-tubes were obtained. Individual sieve-plates were outlined, the pores were counted by means of camera lucida drawings and their areas measured from photomicrographs (plate II).

Knowing the volume delivered through a measured cross sectional area in a given time it is possible to calculate by Poiseuille's formula the pressure gradient required. The calculations will be found in a later section; it suffices here to give the value obtained, 20 atmospheres per meter in order to emphasize the lack of agreement with EWART's figures. There is serious divergence between these two sets of data. If the seive-tube elements in EWART's stems were around 0.25 mm. in length as they were in these then there should be 2000 sieve-plates *per sieve-tube* in 50 cm. of stem. On the other hand, if his rate was 5 mm. per minute through the total phloem (it was almost double that in this case) it would have been about 150 times that or 75 cm. per minute *through the pores.* If any of the rates calculated by BIRCH-HIRSCHFELD, DIXON, MASON, or MÜNCH are multiplied by a factor which will convert them to rates through the sieve-pores and the pressures required to produce those rates calculated, the objections raised to this type of conduction will be obvious.

Granting that these criticisms are valid there remains to be found a mechanism capable of transporting the quantities of material which must move in order to provide for storage, growth, and other life processes.

The proposed mechanism

The fact that carbohydrates are synthesized in the leaves of the higher plant and that utilization of these materials takes place throughout the plant at points more or less remote from that of synthesis suggests that a maintained concentration gradient of osmotically active solutes may exist between the points of synthesis and utilization. The exudation system proposed by PFEFFER and diagramed by BLACKMAN (5) (fig. 1) also

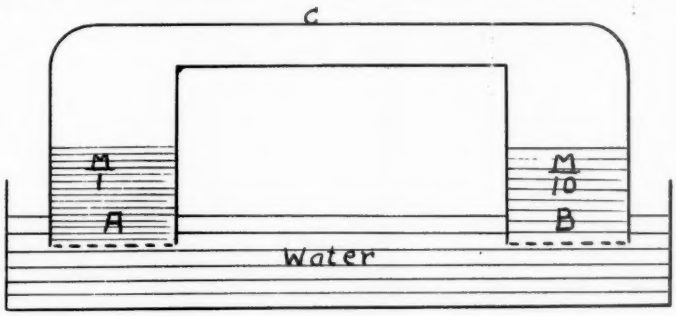

FIG. 1. Exudation system, after PFEFFER and BLACKMAN.

depends upon a maintained concentration gradient, and the continuous dilution and transport of solute in this system indicated the possibility that such a mechanism may take part in the transport of sugars within the plant.

As BLACKMAN explains this mechanism, the arm A is filled with a concentrated sugar solution, arm B contains a less concentrated sugar solution, and the connecting tube C is occupied by water. A and B are closed at their lower ends by semipermeable membranes which are bathed on the outside by pure water. Intake of water at A develops a hydrostatic pressure throughout the closed system which is greater than the maximum osmotic pressure of the solution in arm B and consequently a flow of water into A, along the tube C and out of B, takes place, carrying with it sugar from A and accumulating it at B. To maintain flow through this system the concentration at A must be maintained higher than that at B by supplying sugar to A and removing it from B. The essential point for the present purpose is that this arrangement provides a mechanism for movement of solute by osmotic action from end to end of an open conduit requiring semipermeable membranes only at the two ends.

MÜNCH applies such a system to the plant comparing A to the assimilating leaf cell, B to the growing or storing cell in the stem or root, and the connecting tube C to the sieve-tube. He pictures the movement of sugar from the leaf cells to sieve-tubes and from the latter to the cambium as taking place along plasmodesma, avoiding the plasma membrane, which part of the protoplast he considers impermeable to this material. In his diagram of this system in the plant (fig. 2) he assumes that sugar Z formed

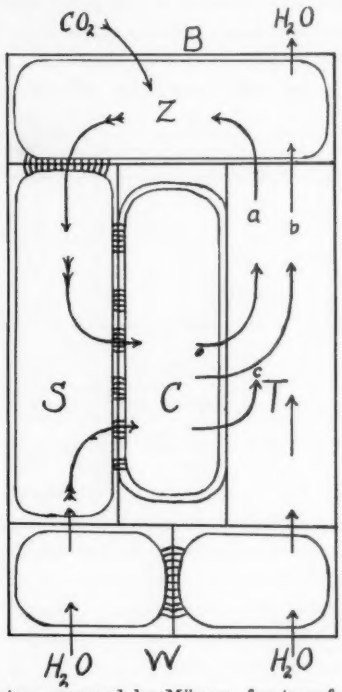

FIG. 2. System proposed by MÜNCH, for transfer of sugar.

in the leaf cell B from CO_2 and water attracts water osmotically from the xylem T, the turgor increasing until sugar solution is forced out along the plasmodesma finally entering the sieve-tube S where most of the longitudinal transfer takes place. From here it travels by way of plasmodesma into the cambium where it is utilized in growth and loses its osmotic activity. The accompanying water which remains is forced into the xylem and reenters the transpiration current which is being drawn in through the roots. Sieve-tubes in the roots due to their sugar content absorb water osmotically from the soil through the intervening cells and by the same

utilization in the cambium an exudation into the xylem takes place and root pressure is produced.

There can be no doubt that the osmotic system proposed by these men would function in the translocation of solutes in the plant if all of the necessary conditions exist. The most obvious criticism of MÜNCH's proposition is the objection to conduction through the sieve-tube, which was raised in the previous section. The limitation of organic transport to plasmodesma and sieve-pores would lower the rates to values incompatible with those calculated as necessary by DIXON and others.

The mechanism proposed in the present paper is similar to that of MÜNCH except that in place of the sieve-tube with its lining of protoplasm, the total phloem, limited by the cambium or similar semi-permeable layers, is suggested as the channel for longitudinal movement.

In the diagram (fig. 3) L_1, L_2 and L_3 are leaf cells. X represents the

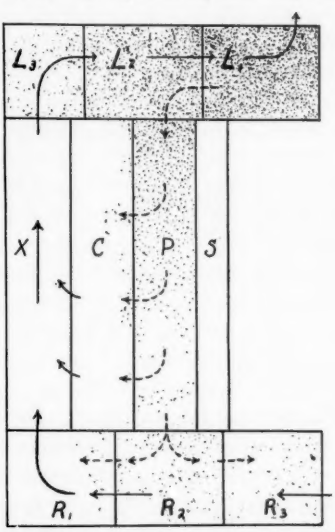

FIG. 3. System proposed to account for translocation of sugar in the phloem.

water conducting elements of the xylem, C the cambium, P the phloem, S the external limiting layer and R_1, R_2 and R_3 root cells. The solid arrows represent the moving transpiration water, the broken ones the flow of sugar solution. The amount of stippling indicates the concentration of sugar at different levels.

The way in which sugar is delivered into the phloem is not being considered in this paper, but certain facts concerning this process cannot be disputed. Of the sugar synthesized in the leaf a major portion passes to

the vascular strands and is eventually transported to remote parts of the plant. MÜNCH has pointed out that because of the volume relationships of the leaf a movement of only 2 μ to 3 μ per hour is required to account for the passage of sugar from leaf cells to vascular tissue while much higher rates occur in the more constricted petiole and stem. Since the intercellular spaces in the leaf do not become injected with solution, or filled with crystalline solids, it seems reasonable to assume that passage takes place from cell to cell and that sugar solution is delivered to the phloem under pressure. Here, because of absorption by living cells of the phloem, cortex, and cambium and a certain loss along the rays into the wood parenchyma, the concentration is gradually diminished. Water remaining after utilization or storage of the carbohydrates and vacuolation of newly formed cells is forced into the xylem and a mass flow in the direction of lowering concentration results. A dynamic equilibrium is set up across the cambium, the relative pressure in the phloem as compared with the xylem being determined by the concentration of solutes, and the absolute pressure by the availability of water.

In examining the plant for evidence of such a mechanism, the presence of exudation pressure in the phloem is quite significant. When the stem of an active plant is dissected sap will be seen to flow from the phloem, while a subatmospheric pressure is found in the xylem if transpiration is taking place. The chlorenchyma may also exhibit a slight exudation but the pith and cortex have no tendency to lose sap. Intercellular spaces in these latter tissues are air-filled and quite prominent; but in the phloem they are lacking, or if present they are filled with solution. The cambium or a layer of small densely filled cells separates phloem and xylem, and across this layer a pressure gradient of several atmospheres often exists. On its outer side the phloem is limited by a starch sheath, endodermis, cork cambium or a layer of disintegrating phloem having air-filled intercellular spaces. These layers by virtue of active protoplasts, and either differences in degree of condensation of cell wall material, or limited available wall area, are able to confine the phloem sap and prevent its loss under pressure.

The pressure gradient between the phloem and xylem may be explained on the basis of known concentrations in these two tissues, the cambium acting as a layer of pure protoplasm exhibiting its characteristic property of semipermeability. KRAUS (**41**) found the sap exuded from the phloem of cucurbit fruits to contain an average of 8.7 per cent. of solids (as calculated from 17 determinations) of which as high as two-thirds might be sugar. In the present work one set of twenty-one collections from cucurbit stems gave an average dry weight percentage of 8.9, another set of twenty collections taken later in the season averaged 9.8 per cent. dry weight. MÜNCH found the concentration of water soluble materials in the sap

exuded from the bark of several species of trees to range from 14 to 31 per cent. Concentrations of xylem sap as determined by DIXON and ATKINS (**19**) and others are always much lower than this at the time when assimilation and downward translocation are occurring. On the other hand, DIXON'S (**18**) osmotic pressure determinations on leaves indicate concentrations as high as one molal. Thirty-one determinations gave an average value of 14.5 atmospheres or roughly two-thirds molal in terms of sugar. RUHLAND (**57**) found a concentration in palisade cells isotonic with 0.8 molal sucrose. Data collected in this laboratory on the sap expressed from pear leaves indicate concentrations approaching one molal of undissociated solute. Hexose solutions of this range of concentrations would contain from 10 to 15 per cent. solute and each gram of sugar would be associated with from 6 to 10 cc. of water occupying corresponding volumes in the leaf. When this amount of sugar has been condensed to starch or cellulose it occupies about 0.63 cc. the associated water having been almost entirely lost. It seems reasonable to assume, therefore, that the mechanism diagramed in figure 3 actually occurs in the plant, longitudinal movement taking place through the phloem.

Considering now the flow of solution within this tissue there appear to be four possible channels through which the substances may pass. These are (1) the lumina of the sieve-tubes, (2) the parenchyma cells, lumina and end walls included, (3) the walls of part or all of the phloem cells, and (4) intercellular spaces. There is no reason for assuming that all of the movement need be confined to any of these channels since the pressure gradient responsible for movement may exist throughout the space confined by the semipermeable limiting layers. The comparative importance of the different channels, therefore, depends primarily upon their relative resistance to mass flow of sugar solution and upon this basis it seems reasonable to conclude that the first two mentioned channels take little part in actual transport. In addition to the objections raised in the previous section, the movement of sugar into and out of the living cell with sufficient rapidity is a serious problem. Plasmolysis (**57**) and absorption (**35, 36**) experiments indicate that absorption of sugar by living cells is a slow process. Loss of sugar from healthy cells seems to be even more difficult to explain (**36, 64**). In the photosynthesizing cell we have an exception to this rule since a source of sugar is present within the protoplasm but RUHLAND'S results and those obtained in this work indicate that phloem cells are not an exception.

Intercellular spaces were few in the plants studied but where present they constitute open channels for passage. Old sieve-tubes which have lost their protoplasmic linings may also conduct but they are seldom found in active phloem. There remains the possibility that mass flow may take

place along the phloem walls. Evidence suggesting that this is the case will be presented in the following section.

Experimental results and calculations on the rates of movement of materials in the phloem

Evidence in support of the proposed type of mechanism for translocation of organic nutrients in the plant should include data on the nature of the phloem walls and a determination of their capacity to conduct. The most obvious objection to this mechanism is that phloem walls are too thin, occupying too small a proportion of the total volume. This impression may be the result of studying dehydrated mounted sections of phloem (plate IIIb). The drawings of FISHER (**25**) and HILL (**33, 34**) and the illustrations used in this work (plates IIIa, IVb and Va) show that phloem walls are relatively thick in their natural condition. Their loss of volume upon drying (plates IVa and Vb) indicates a high water content.

The readiness with which molecules of sugars, electrolytes and certain vital stains diffuse through cellulose walls demonstrates their high permeability as compared with that of the protoplasmic membranes which they enclose. Experiments by RUHLAND (**57**) and others indicate that phloem walls are no exception to this rule. PRIESTLEY (**56**) finds phloem walls to be of "amyloid" nature and free of fats. He emphasizes the relation of cell walls to the conduction of nutrients in the nutrition of the growing point. Results of the present work give additional information on this subject.

The stolon of a potato weighing 210.3 grams was sectioned, the sections mounted in tap water (plate IIIa) and ten fields, chosen at random throughout the phloem, were drawn by means of the camera lucida. Similar sections were stained in safranin in 50 per cent. alcohol, dehydrated, mounted in balsam (plate IIIb) and drawn in the same way. Other sections (plate IVa) were treated on a slide with 95 per cent. alcohol to kill them, a cover glass clamped over them to keep them flat, and dried first in a 50° oven and then in an 80° oven. The drawings from these three sets of slides were cut out and weighed and the wall area calculated as percentage of the total area. Representative fields are shown in plates IIIa, IIIb, and IVa.

These figures showing the shrinkage in only two dimensions indicate a water content constituting at least 50 per cent. of the wall volume. An observed longitudinal shrinkage of 15 per cent. and the possibility that an open structure with air spaces exists in the dry walls allow for an even greater water content.

In order to study the rate of movement of organic materials through this stolon and the magnitude of pressure gradients, projections were made of transverse sections at a magnification of 200x and the areas calculated.

TABLE I

WALLS OF THE PHLOEM OF POTATO STOLON EXPRESSED AS PERCENTAGE OF TOTAL CROSS-SECTION OF THE PHLOEM

FIELD	FRESH	DEHYDRATED IN ALCOHOL	AIR DRIED
	per cent.	*per cent.*	*per cent.*
1	40.5	25.7	16.6
2	37.0	24.3	18.6
3	36.5	25.8	18.4
4	37.3	21.7	18.9
5	36.4	30.5	17.7
6	33.9	28.0	18.6
7	39.1	26.5	18.8
8	36.6	23.1	20.8
9	37.1	23.8	16.4
10	33.3	28.9	19.9
Average	36.8 ± 0.44	25.8 ± 0.55	18.5 ± 0.32

If the data given by C. F. CLARK (United States Department of Agriculture Bulletin 958) on the rate of development of tubers are plotted, a daily increment of fresh weight of 5 grams per day will be found during the period of optimum growth. The data given by C. H. JONES and B. O. WHITE (The Report of the Chemists in the 13th Annual Report of the Vermont Experiment Station, 1899–1900) indicate that mature potatoes have a dry weight of 18 to 20 per cent. The 210.3 gram tuber in this experiment had a dry weight of 16.7 per cent. Multiplying the 5-gram increment by 0.20 we find a 1-gram daily increment in dry weight and if we accept JONES and WHITE's analyses approximately 80 per cent. of this

TABLE II

AREAS OF MEASURED POTATO STOLON

	*mm.*²	*mm.*²
Cortex	2.49	
External phloem	1.16	1.16
Xylem	2.74	
Internal phloem	0.27	0.27
Pith	0.39	
Total	7.05	1.43 sq. mm.

is carbohydrate giving a daily increment of 0.8 gram of carbohydrate. Expressing this as a 10 per cent. solution gives us 7.2 cc. per day through 1.43 sq. mm. or $7.2 \div 0.0143 = 503.5$ cm. per day linear rate of flow, or $503.5 \div 24 = 21$ cm. per hour through the phloem, and $21 \div 0.368 = 57$ cm. per hour through the walls. This rate of flow through the walls is about the same as that calculated by DIXON for flow through the total phloem. Since no information is given as to how he treated the section of stolon which he measured, the lack of agreement cannot be explained. Rapid dehydration of sections often leads to a disproportionate shrinkage of the phloem area compared with the remainder of the section. Substituting this value in Poiseuille's formula as given by STAMM (**63**) we obtain a value for the pressure gradient required to deliver the solution through the walls at the rate required.

$$P = 8\frac{Rnl}{\pi r^4}.$$

P = pressure in dynes per cm.^{2}
R = rate of flow in cc. per sec.
n = viscosity of a 10 per cent. solution of sucrose.
l = length of gradient or tube in cm.
r = radius of conducting element in cm.

To use linear rate R_1 we substitute for R, $R_1 \times$ area A; $P = 8\frac{R_1 nAl}{\pi r^4}$; the formula becoming $P = 8\frac{R_1 nl}{r^2}$. R_1 in this case $= 57 \div 3600 = 0.0158$ cm. per sec.; $n = 0.015$; $l = 1$.

$\pi r^2 = 1.43 \times 10^{-2} \times 0.368 = 0.00527$ cm.^{2}

$r^2 = 0.00168$ cm.^{2}

$P = 8 \times \frac{0.0158 \times 0.015}{0.00168} \times 1 = 1.13$ dynes per cm., 0.00115 g. per cm. or 1.1×10^{-6} atmospheres per centimeter.

This is the pressure gradient required to deliver the solution through the total wall area if it acted as a single pore. This value would be four times as great or 4.5×10^{-6} atmospheres per centimeter if only the space occupied by water of hydration were available. There might reasonably be expected a pressure fall of 4 atmospheres in the average stolon 5 cm. in length. This would be 0.8 atmosphere per centimeter and $0.8 \div 1.1 \times 10^{-6} = 7.15 \times 10^5$ the factor representing the possible difference in resistance between the walls acting as a single open pore and acting as they exist in the plant.

The surface presented by the cellulose molecules and the included cells is an unknown quantity in this calculation and the resistance offered can only be roughly estimated as by the calculation just presented.

In the next experiment twenty collections of sap were made from as many cucurbit stems, as previously described (page 6). The results are

TABLE III

WEIGHT OF SAP, PHLOEM AREA AND RATE OF LONGITUDINAL SAP FLOW IN CUCURBIT STEMS

STEM	ENDS	TIME	SAP		DRY AS PERCENTAGE OF FRESH WT.	PHLOEM AREA	SAP PER END PER MINUTE	LINEAR RATE OF FLOW PER MINUTE
			FRESH WT.	DRY WT.				
no.	*no.*	*min.*	*gm.*	*gm.*	*per cent.*	*cm.²*	*cc.*	*cm.*
1	1	2	0.0443	0.0041	9.2	0.144	0.0221	0.15
2	1	2	0.0592	0.0035	5.9	0.133	0.0296	0.22
3	1	1	0.1040	0.0111	10.7	0.064	0.1040	1.62
4	1	1	0.0409	0.0039	9.5	0.064	0.0409	0.64
5	2	1	0.1360	0.0118	8.7	0.100	0.0680	0.68
6	2	1	0.1328	0.0149	11.2	0.087	0.0664	0.76
7	1	1	0.1478	0.0139	9.4	0.181	0.1478	0.82
8	2	1	0.1517	0.0172	11.3	0.088	0.0759	0.86
9	1	1	0.0414	0.0046	11.1	0.075	0.0414	0.55
10	1	1	0.0584	0.0053	9.1	0.033	0.1168	3.54
11	1	½	0.0182	0.0021	11.5	0.017	0.0182	1.07
12	1	1	0.0317	0.0036	11.3	0.024	0.0317	1.32
13	1	1	0.0313	0.0041	13.1	0.029	0.0313	1.08
14	1	1	0.0264	0.0027	10.2	0.015	0.0264	1.76
15	1	1	0.0353	0.0029	8.2	0.041	0.0353	0.86
16	1	1	0.0838	0.0080	9.5	0.105	0.0838	0.80
17	1	2	0.1408	0.0131	9.3	0.077	0.0704	0.91
18	1	2	0.1062	0.0110	10.4	0.092	0.0531	0.57
19	1	2	0.1397	0.0131	9.4	0.058	0.0699	1.20
20	1	2	0.0916	0.0082	8.9	0.028	0.0458	1.64
			1.6215	0.1591		1.455	1.1788	

given in table III. Stems no. 1 and 2 were from an old plant starting to dry up. No. 10 was the tip of a young rapidly growing stem.

If the number of cubic centimeters, from 20 ends for one minute, be divided by the total area of the phloem in the 20 ends, we get the average

TABLE IV

PHLOEM WALL AREAS IN CUCURBIT STEMS

SECTION	CONDITION	WALL AREA PERCENTAGE OF TOTAL	PERCENTAGE OF ORIGINAL
A	Fresh	28.5	
A	Dry	14.6	13.2
B	Fresh	29.8	
C	"	27.5	
D	"	30.6	
E	Dry	14.4	

length of phloem which would have to have its volume displaced each minute in order to provide the volume collected. That is, $1.1788 \div 1.455 = 0.81$ cm. linear rate of flow. Fresh sections mounted in tap water, and air dried sections were used to estimate wall areas by camera lucida drawings. Section A (plate Va) was mounted fresh and a field drawn, then air dried (plate Vb) and the identical field redrawn. These fields were all drawn from large bundles. In the small bundles the sieve-tubes are smaller in diameter and the walls would occupy even a larger percentage of total area. For purposes of calculation we will assume that the walls occupy 30 per cent. of the total area when wet and 15 per cent. when dry.

Sixteen average sieve-plates were drawn with the camera lucida so that the exact number of pores might be determined.

TABLE V

SIEVE-PLATE AREAS AND AREA PER PORE VALUES IN CUCURBIT STEMS

SIEVE-PLATE	NUMBER OF PORES	AREA OF SIEVE-PLATE	AREA NUMBER OF PORES
		$cm.^2 \times 10^{-5}$	$cm.^2 \times 10^{-6}$
1	37	1.965	0.531
2	42	2.263	0.540
3	45	1.965	0.437
4	46	1.208	0.263
5	50	1.058	0.211
6	56	3.465	0.620
7	62	2.420	0.391
8	63	3.020	0.480
9	69	2.567	0.373
10	71	3.020	0.426
11	71	3.780	0.532
12	75	3.175	0.424
13	76	3.020	0.397
14	97	3.175	0.327
15	108	3.175	0.294
16	113	3.465	0.267
Total	1,081	42.741×10^{-5}	

Dividing 42.74×10^{-5} by 1081 we find that each pore is allotted an averaged area of 0.395×10^{-6} cm.^{2} or 39.5 μ^2.

The pores in these plates were from 1.25 μ to 1.75 μ in diameter and the plates varied from 3 μ to 10 μ in thickness with an average of about 5 μ.

A number of phloem groups were projected at a magnification of 200 times. These sections were all mounted in tap water, a few having been

stained in dilute acid violet solution first. The figures in table VI give an idea as to the percentage of phloem area occupied by sieve-tubes.

TABLE VI

PERCENTAGE OF PHLOEM OCCUPIED BY SIEVE-TUBES IN CUCURBIT STEMS

STEM	OUTER GROUP		INNER GROUP	
	NUMBER OF TUBES	AREA PERCENTAGE OF TOTAL	NUMBER OF TUBES	AREA PERCENTAGE OF TOTAL
1	131	21.2	110	23.6
2	88	18.1	80	18.1
3	54	10.7	70	19.4
4	53	18.9	52	21.6
5	41	14.3	36	14.8
6	79	13.4	101	20.7
7	85	20.8	122	27.9
8	140	16.8	121	19.7
9	73	14.4	73	18.1
10	98	18.9	57	23.0
11	51	19.1	40	22.6
12	33	13.2	44	13.2
13	55	15.3	52	18.1
14	34	15.5	48	19.1
15	42	15.0	40	16.6
16	74	19.5	84	23.0
17	109	16.2	93	15.6
18	30	17.1	42	18.1
19	66	13.4	73	12.0
20	72	14.1	74	13.1

If we may assume that the sieve-tubes occupy 20 per cent. (**45**) of the total phloem area we have the data from which to calculate the pressure gradient necessary to deliver through the sieve-tubes and through the walls, the volume of sap collected. Using EWART's figure, 2 μ, for the diameter of pores we find the area of the pore to be $3.14\ \mu^2$, and this represents $3.14 \div 39.5 \times 100$ or 7.95 per cent. of the area of the sieve-plate.

The linear rate of flow through these pores $= 0.81 \div 0.20 \div 0.0795 = 51$ cm. per min. or 0.85 cm. per sec. $= R_1$.

$n = 0.015$

$l = 5 \times 10^{-4}$ cm.

$r = 1 \times 10^{-4}$ cm.; $r^2 = 1 \times 10^{-8}$ cm.^{2}

$$p = \frac{8 \times 0.85 \times 0.015 \times 5 \times 10^{-4}}{1 \times 10^{-8}} = 5100 \text{ dynes},$$

5.2 g, or 0.005 atmosphere pressure gradient to maintain flow through one

set of sieve-plates. A measure of 74 sieve-tube elements in these stems gives a value 0.26 mm. average length, that is, about 4 sieve-plates per mm., 40 per cm., or 4000 per meter. Thus it would necessitate a pressure gradient of 20 atmospheres per meter to maintain this rate of flow in these stems. If all of the dry weight of the sap were sucrose a maximum osmotic pressure of but 8 atmospheres could be developed, which would be inadequate to cause the observed exudation. In view of the rates of from two to four times the above value which have been observed, and also of the fact that the pores seem to be filled with protoplasm rather than open, or at least obstructed by a cellulose lamella, it would seem impossible to explain the exudation on the basis of mass flow through the sieve-tubes.

Examining now the possibility for movement through the walls the linear rate per cm. would be:

$R_1 = 0.81 \div 60 = 0.0135$ cm. per sec. through the phloem
$= 0.045$ cm. per sec. through the phloem walls
$n = 0.015$; $l = 1$ cm.

$\pi r^2 = 1.455 \div (20 \times 20) \times 0.3 = 0.00109$ cm.^{2} for walls per phloem group and $r^2 = 0.000348$ considering the walls of each phloem group as if they formed a single capillary tube. Then $p = \frac{8 \times 0.045 \times 0.015}{0.000348}$, $= 15.5$ dynes per cm. or 1.5×10^{-5} atmosphere per cm. gradient through the walls of 20 phloem groups, there being 10 bicollateral bundles in the internode. If the same calculation is made using 15 per cent. of the total area as occupied by the free space filled with solution in the walls the value 6.1×10^{-5} atmosphere per cm. is obtained. It seems probable, therefore, that the greater portion of the exuded sap from cucurbit stems flows out through the walls rather than through the sieve-tubes.

Another series of collections was made over longer time intervals and the following data collected.

Dividing the total volume 1.2589 cc. by 0.0918 we find that a volume of solution equal to the volume of the total phloem in 13.7 cm. of stem flowed out during the 21 minutes, about 1 cm. of stem having been removed during the collection.

These data bring out two interesting points in this work. The proteinaceous material in the cucurbit sap gelatinizes rapidly upon exposure to air and tends to clog the ends of the cut stems. The removal of a thin section allows flow to be resumed at about its former rate. This rate, however, drops off rapidly at first, and then more slowly, approaching asymptotically a value of about 0.3 cm. per minute.

The peduncle of a mature pumpkin was sectioned as was the stem below and above the fruit.

TABLE VII

SAP FLOW FROM CUCURBIT STEMS

COLLECTION NUMBER	FRESH WEIGHT OF SAP	DRY WEIGHT OF SAP	DRY WEIGHT PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR RATE OF FLOW	TIME	RATE $\frac{\text{CM.}}{\text{MIN.}}$
Collection A				Pumpkin stem			
				*cm.*²	*cm.*	*min.*	
1	0.1190	0.0101	8.5	0.173	0.688	1	0.688
2	0.0660	0.0046	7.0		0.381	1	0.381
3	0.0800	0.0056	7.0		0.463	2	0.231
4	0.0465	0.0031	6.7		0.269	4	0.067
Collection B	Large pumpkin stem.		Collection made for 5 minutes from both ends				
	0.9484	0.0878	9.2	0.175	5.43	5	0.543
Collection C	Peduncle of a growing pumpkin						
1	0.1683	0.0147	8.7	0.0918	1.833	1	1.833
2	0.0593	0.0041	6.9		0.646	1	0.646
3	0.1005	0.0074	7.4	*	1.094	1	1.094
4	0.1028	0.0081	7.9	*	1.120	1	1.120
5	0.0916	0.0070	7.6		0.997	1	0.997
6	0.0575	0.0042	7.3		0.627	1	0.627
7	0.1404	0.0103	7.3	*	1.530	2	0.765
8	0.1971	0.0158	8.0		2.240	4	0.560
9	0.2074	0.0165	8.0	*	2.800	5	0.560
10	0.1340	0.0099	7.4	*	1.460	4	0.365

* This stalk was cut 5 times during the collection, thin sections about 1–2 mm. in thickness being sliced off with a safety razor blade. Collection made from stem end.

The pumpkin weighed 18,940 gm. of which 9 per cent. was dry weight. If this fruit were made from a 9 per cent. solution in 100 days then 189.4 cc. flowed in per day, 7.9 cc. per hour or 0.13 cc. per minute. This divided by 0.450 gives a linear rate of 0.292 cm. per min. The smaller size of the

TABLE VIII

AREAS OF PEDUNCLE AND STEM OF PUMPKIN

TISSUE	PEDUNCLE	STEM ABOVE	STEM BELOW
	*cm.*²	*cm.*²	*cm.*²
Phloem	0.450	0.084	0.240
Xylem	0.197	0.042	0.154
Ground tissue	3.160	0.800	1.017

developing peduncle and the difference in gradient and viscosity between day and night and bright and dull days would necessitate a somewhat greater rate than this during part of the time at least. The general agreement in this figure indicates, however, that rates of from 0.3 to 0.5 cm. per minute through the phloem of these stems may be expected. Since the pressure gradient necessary to produce rates through the sieve-tubes proportional to these rates through the phloem cannot be accounted for by the concentrations found in these elements it seems much more likely that. at least in the case of cut stems, the observed volumes of solution moved out through the walls. The gradient necessary to produce a linear rate of movement of 0.3 cm. per minute would be around 1.14×10^{-5} atmosphere per cm. for movement through the phloem walls acting as a single capillary. An actual gradient of about 0.05 atmosphere per cm. might be expected. giving a factor of 4400 to represent the difference in resistance between the phloem walls, acting as a single capillary, and acting as they do in the plant.

MÜNCH (**48**) has given a value for the linear rate of flow necessary to provide for growth in several forest trees. Assuming movement to take place through the sieve-tubes, he calculated a rate of from 16 to 31 cm. per hour for an average 120 day growth period. The walls may well occupy as much or more space than the sieve-tubes in these stems, so comparable rates would probably occur if they were the channels. Rates of from 2.81 to 4.46 per hour occurred in the stems of fruits.

Calculations based on data collected in this laboratory on the rate of increase of dry weight in the pear tree give comparable rates. A group of 16 two-year-old trees of uniform size and shape were used. They were taken out two at a time and analyzed. The following data have been selected and tabulated (table IX). The last four columns give values for the osmotic pressure at 0° C., calculated from the freezing point lowering of expressed sap of the bark.

The average diameter at the top of the trunk for these trees was 3.10 cm. Measurements were made of the thickness of phloem and cortex of seven trunks of this diameter. Strips of bark were taken from the north and south sides of the trunks and microtome sections of these were projected at a magnification of 100x, outlined and measured. Table X gives the data on these. In table XI the cell wall : phloem area ratios obtained from camera-lucida drawings of these sections are given. The average percentage of the phloem area occupied by walls in the fresh sections was 40.54 per cent. and in the air dried sections 23.50 per cent. The average thickness of cortex was $\frac{275.7}{190}$ or 1.45 mm. and of phloem $\frac{84.89}{255}$ or 0.33

TABLE IX

COMPOSITION OF PEAR TREES REMOVED AT INTERVALS THROUGHOUT THE YEAR

Tree	Date removed	Trunk and roots			Sap concentration Osmotic pressure at 0° C.			
		Dry weight	Starch	Diameter at top of trunk	Leaves	Branches (bark)	Trunk (bark)	Roots
no.		*gm.*	*per cent.*	*cm.*	*atm.*	*atm.*	*atm.*	*atm.*
1393	2/13	854.4	8.19	2.50	—	17.81	20.45	12.40
1383	2/15	756.6	7.01	2.88	—	20.42	20.58	13.72
1398	3/14	881.1	8.22	2.69	—	16.65	17.70	12.68
1377	3/18	729.8	7.47	2.57	—	15.19	18.05	12.60
1354	4/22	800.4	6.04	2.52	11.68	11.55	12.64	11.59
1330	4/24	618.4	2.91	2.90	13.97	9.82	10.71	10.59
1352	6/9	751.0	3.18	3.00	16.18	10.92	9.27	9.90
1327	6/12	755.2	5.84	2.92	23.82	15.28	9.75	9.66
1334	7/30	772.3	10.36	3.25	22.42	12.08	9.14	10.71
1358	8/4	862.7	9.98	3.15	24.21	12.42	10.48	11.28
1309	10/13	1187.3	12.41	2.45	22.20	14.17	13.42	11.71
1284	10/20	1439.9	12.12	3.30	28.07	17.64	15.76	12.10
1305	12/17	1446.7	9.76	3.68	48.50	19.64	17.88	14.08
1331	12/18	1990.3	12.86	4.20	—	20.80	18.96	13.81
1280	2/24	1749.5	7.85	3.92	—	18.59	20.21	13.35
1255	2/26	1792.5	9.22	3.70	—	19.56	18.78	13.12

mm. Denoting the total radius as r, the cortex thickness as c, and the phloem thickness as p, the area of the phloem would be

$$\pi (r-c)^2 - \pi [r-(c+p)]^2; r = 1.55 \text{ cm.}$$

$c = 0.145$ cm., $p = 0.033$ cm. and the phloem area is 0.30 cm.^{2}

From the data of MÜNCH (**48**) and CHANDLER (**10**) and the osmotic concentrations recorded in table IX it seems reasonable to assume a concentration of about two-thirds molal in these tissues. CHANDLER (**10**) finds a theoretical molecular weight of about 200 for these solutes and the vertical gradients of sucrose and reducing sugars found by MASON and MASKELL (**47**) indicate that the phloem sap should at least not fall below this average.

A two-thirds molal solution of a substance of molecular weight 200 would have a percentage composition of 11.77 per cent. solute. Table IX shows that between August 1 and December 18 there was a daily increase in dry weight of 6.53 grams per tree which at the above concentration would correspond to a volume of 55.6 cc. flowing daily or 2.32 cc. per hour. This volume through 0.30 cm.^{2} would require a linear rate of 7.73 cm. per hour through the total phloem or about 19 cm. per hour through the walls and would necessitate a pressure gradient of 1.60×10^{-8} atmosphere per centi-

TABLE X

THICKNESS OF PHLOEM AND CORTEX OF PEAR BARK

SAMPLE NO.	PHLOEM			CORTEX		
	NUMBER OF MEASUREMENTS	TOTAL THICKNESS	AVERAGE THICKNESS	NUMBER OF MEASUREMENTS	TOTAL THICKNESS	AVERAGE THICKNESS
		mm.	*mm.*		*mm.*	*mm.*
1N	25	6.57	0.263	13	18.8	1.45
1S	20	4.37	0.218	7	10.9	1.56
2N	22	6.30	0.286	14	13.9	0.99
2S	25	9.76	0.391	15	14.3	0.94
3N	15	7.37	0.491	16	22.1	1.38
3S	13	6.20	0.477	12	20.2	1.68
4N	17	6.13	0.361	14	20.0	1.43
4S	20	6.17	0.308	15	22.1	1.47
5N	16	4.72	0.295	16	22.3	1.39
5S	16	5.18	0.323	15	20.0	1.33
6N	18	5.83	0.324	14	24.0	1.72
6S	17	6.69	0.393	13	22.7	1.75
7N	17	4.80	0.282	14	23.4	1.67
7S	14	4.80	0.343	12	21.0	1.75
Total	255	84.89		190	275.7	

TABLE XI

CELL WALL : PHLOEM AREA RATIOS OF PEAR BARK

SAMPLE NO.	WEIGHT			WALLS, PER CENT. TOTAL PHLOEM
	WALLS	LUMINA	TOTAL	
	gm.	*gm.*	*gm.*	*per cent.*
1 N a	2.16	2.97	5.13	42.1
b	1.51	2.21	3.72	40.6
1 S a	2.23	2.85	5.06	43.8
b	1.42	1.91	3.33	42.7
2 N a	1.79	2.96	4.75	37.7
b	2.81	4.74	7.55	37.2
2 S a	1.85	2.70	4.55	40.7
b	2.18	3.06	5.24	41.7
3 S a	2.97	5.14	8.11	36.6
b	2.82	4.39	7.21	39.1
4 Fresh	2.01	2.41	4.42	45.5
Air dried	1.30	3.69	4.99	26.2
5 Fresh	2.85	4.49	7.34	38.8
Air dried	1.54	5.86	7.40	20.8

meter for the total phloem walls acting as a single capillary. Assuming a gradient of 0.05 atmosphere per centimeter possible in the tree, a factor 3.1×10^6 represents the relative resistance of the walls compared to a single capillary of equal cross sectional area.

Rates of flow through petioles have been calculated by both DIXON (**21**) and BIRCH-HIRSCHFELD (**4**). In order to check DIXON's rate, five leaves of *Tropaeolum majus* were measured and fresh sections of the petioles were projected, the phloem groups outlined, and the areas determined with the planimeter. Using the assimilation rate which DIXON quotes from BROWN

TABLE XII

AREAS OF LAMINA AND OF PETIOLE PHLOEM OF *Tropaeolum* LEAVES

LEAF	LAMINA AREA	AREA OF PHLOEM OF PETIOLE
	*cm.*²	*cm.*²
1	71.9	0.00476
2	70.5	0.00371
3	75.0	0.00345
4	75.5	0.00394
5	62.8	0.00359
Totals	355.7	0.01945

and MORRIS these leaves would form 0.03557 gram per hour or 0.3557 gram in 10 hours and two-thirds of this or 0.3271 gram would be transported through the petiole in 24 hours. Using the factor 10 to arrive at a volume, 2.371 cc. would pass through 0.01945 cm.² of phloem in 24 hours at a linear rate of $\frac{2.371}{0.01945 \times 24}$ or approximately 5.1 cm. per hour. The walls were not measured in this case but 30 per cent. would be a conservative estimate, and through this area the rate would be 17.0 cm. per hour.

BIRCH-HIRSCHFELD used the leaf of *Phaseolus multiflorus* in her calculation. She assumed an hourly loss of 0.28 gram from 1 sq. meter and $\frac{0.28 \times 117}{100^2}$ or 0.003276 gram per hour from her leaf. Through the 0.004598 cm.² of phloem this would be 0.71 cm. per hour for the pure solute, or 7.1 cm. per hour for about a 10 per cent. solution. Through the 37.2 per cent. of the phloem occupied by walls this would be 19.1 cm. per hour. A *Phaseolus* leaf which was measured to check this result had a lamina of 64 cm.² and the phloem measured 0.0032 cm.² Using the same rate of loss from the leaf, a flow of 5.58 cm. per hour through the total phloem, or 15 cm. per hour through the walls, would be adequate.

SACHS (**59**) found a rate of assimilation of 1.8 grams per square meter per hour in *Helianthus annuus*. With this same plant BROWN and MORRIS (**7**) give rates around 1 gram per square meter per hour. BROWN and ESCOMBE (**8**), measuring CO_2 absorption, arrive at rates which are less than one-half of this, and THODAY (**66**) using an improved one-half leaf method got an average value of 1.65 grams per square meter per hour.

Among the later workers KOSTYTSCHEW (**40**) recorded rates of assimilation in several species arrived at by measuring CO_2 absorption. His values approximate the early figure of SACHS, and BOYSEN-JENSEN (**6**) using a similar method finds values of from 0.5 to 1.0 gram per square meter per hour. Since measurements of the phloem in the petioles are not available in any of these experiments a set of determinations was made on *Phaseolus* leaves using the stamping method of THODAY (**66**), weighing the changes in dry weight and measuring the phloem in the petioles from projections of sections. The center leaflets of the compound leaves were used, a four square-centimeter area being stamped on each side of the midrib.

The plants were moved from the greenhouse to a dark room at 4:00 P. M. on February 20. The leaves were stamped and tagged between 8:00 and 8:30 P. M. the same evening and the experiment started the following morning. Four sets of ten leaves each were used; eight samples were collected falling into five groups. The four sets will be called ab, ac, cd, and ce, each letter designating a sample. The *a* samples were collected at 8:00 A. M. February 21, after 16 hours in the dark room attached to the plants. The *b* sample was collected at 6:00 P. M. February 21 after 16 hours in the dark room attached to the plant, followed by 10 hours illumination detached from the plant with petioles in water. The *c* samples were collected at 6:00 P. M. February 21, after 16 hours in the dark and 10 hours illumination attached to the plant. The leaves of the *d* sample, after the same treatment as *c*, were detached and the petioles placed in water, the *d* sample being collected at 10:00 A. M. February 22, after 14 hours in the dark. The *e* sample had the same treatment except that these leaves remained attached throughout.

The printed leaf areas were cut out with scissors, placed with a drop of ether in a small test tube, brought to 100° C. in a boiling water bath, dried in a 55° oven for 24 hours and brought to constant weight in a 95° oven. The two *a* samples and the *e* sample were theoretically similar and weighed nearly alike, so their weights were averaged. The three *c* samples were all similar, so their weights were also averaged. The weights were:

Sample	weight of 40 cm.²	
a	0.0773 g.	16 hours in dark
b	0.0912 "	" " " " " + 10 hours illuminated, detached

c 0.0832 g. 16 hours in dark + 10 hours illuminated, attached
d 0.0798 " " " " " " " " " " " + 14 hours dark detached
e 0.0773 g. 16 hours in dark + 10 hours illuminated, attached + 14 hours dark attached

Weight differences per 40 cm.²

b–a 0.0139 g. assimilation in 10 hours illumination, detached leaves
c–a 0.0059 " " " " " " " attached "
c–e 0.0059 " respiration and translocation 14 hours in dark, attached leaves
c–d 0.0034 g. respiration in 14 hours in dark, detached leaves
b–c 0.0080 " translocated during 10 hours illumination, attached leaves
d–e 0.0025 g. translocated during 10 hours dark, attached leaves

The extraordinarily good agreement of these figures is accidental and resulted from the averaging indicated. The general order of magnitude, however, should be reliable. The value 0.0080 g. translocated in 10 hours from 40 square centimeters gives an hourly loss of 0.2 g. per hour per square meter. Table XIII gives the areas of these leaves and table XIV the areas of the phloem in the petioles.

TABLE XIII

AREAS OF *Phaseolus* LEAVES IN SQUARE CENTIMETERS

LEAF	ab	ac	cd	ce
	cm.²	*cm.²*	*cm.²*	*cm.²*
1	33.5	38.4	21.9	26.4
2	40.7	27.1	32.5	26.6
3	33.9	24.8	22.2	38.2
4	35.6	34.5	34.4	22.2
5	44.1	43.0	30.2	35.4
6	24.5	22.0	38.9	25.9
7	20.0	25.8	42.0	37.1
8	22.6	29.4	28.4	34.5
9	23.9	24.6	21.8	35.7
10	26.6	33.9	27.8	34.6
Total	305.4	303.5	300.1	316.6
Average	30.54	30.35	30.01	31.66

The average leaf area was 30.64 cm.², and the average phloem area 0.123 mm.² or 0.00123 cm.² A transport of 0.2 g. per square meter = 0.00002 g. per square centimeter per hour and $30.64 \times 0.00002 = 0.0006128$ g. or 0.006128 cc. of a 9 per cent. solution. $\frac{0.006128}{0.00123} = 5$ centimeters per hour

linear rate of flow through the entire phloem. Table XV gives the cell wall: phloem area ratios of seven of these petioles. Dividing 5 by 0.372 we arrive at a rate of 13.5 cm. per hour through the walls.

TABLE XIV

AREAS OF PHLOEM IN *Phaseolus* PETIOLES IN SQUARE MILLIMETERS

LEAF	ab		ac		ed		ee	
	PHLOEM	PETIOLE	PHLOEM	PETIOLE	PHLOEM	PETIOLE	PHLOEM	PETIOLE
	*mm.*²	*mm.*²	*mm.*²	*mm.*²	*mm.*²	*mm.*²	*mm.*²	*mm.*²
1	0.161	2.07	0.134	1.86	0.106	1.15	0.093	1.19
2	0.208	2.64	0.126	1.63	0.125	1.74	0.132	1.61
3	0.139	1.94	0.078	1.26	0.078	1.01	0.117	1.66
4	0.203	2.64	0.119	1.79	0.137	1.54	0.091	1.03
5	0.252	2.85	0.171	2.05	0.130	1.59	0.150	1.86
6	0.103	1.39	0.098	1.21	0.127	1.96	0.095	1.16
7	0.103	1.08	0.082	1.03	0.147	2.06	0.092	1.08
8	0.112	1.75	0.102	1.25	0.088	1.26	0.125	1.47
9	0.100	1.96	0.096	1.02	0.074	0.88	0.147	1.66
10	0.116	1.66	0.139	1.66	0.104	1.03	0.130	1.87
Total ...	1.497	18.98	1.145	14.76	1.116	14.72	1.172	14.59
Average value..	0.1497		0.1145		0.1116		0.1172	

TABLE XV

CELL WALL: PHLOEM AREA RATIOS OF *Phaseolus* PETIOLES

SAMPLE NUMBER	RATIO EXPRESSED AS PER CENT.
1	41.2
2	34.4
3	36.0
4	34.9
5	34.1
6	40.8
7	38.9
Average	260.3 ÷ 7 = 37.2 per cent.

If the mechanism pictured in the previous section actually acts in the transport of organic materials through the phloem of woody stems it should be possible to force certain solutions through this tissue. Apparatus

was designed which allowed the application of pressure to solutions covering the exposed bark of stems and the rates of movement measured under certain standard conditions. After some experimentation a strong solution of Orange G mixed with Higgin's India Ink was decided upon as an indicating solution. A five-centimeter length of stem about 2 centimeters in diameter was used, a strip of bark one centimeter wide being removed from each end leaving an intact strip 3 centimeters wide in the center. The wood of the upper end was coated with hot paraffin and the piece of stem sealed into a glass tube with plasticine and cotton string. The indicator solution was poured in and pressure applied by means of a plunger in the glass tube. Several species were studied by this method. The maximum and minimum distances were measured, the average was estimated visually.

In all of these tests a standard length of 3 centimeters was used. Preliminary tests showed, however, that the rate was inversely proportional to

TABLE XVI

RATE OF MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF HARDY PEAR STEMS.[1] MARCH 13, 1930

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
atm.	*min.*	*cm.*	*cm.*	*cm.*
1	1	0.50	0.60	1.50
1	1	0.30	0.60	1.50
1	2	0.50	1.00	2.00
1	2	0.50	0.75	1.50
1	4	0.75	1.00	3.00
1	4	0.50	1.00	2.50
1	8	1.00	2.00	3.00
1	8	1.00	2.25	3.00
1	12	3.00	3.00	3.00
1	12	2.50	3.00	3.00
½	4	0.30	0.50	1.00
2	4	1.50	2.00	3.00
3	4	2.00	3.00	3.00
4	3	2.50	3.00	3.00
1 check[2]		0.30	0.50	1.00

[1] The buds were swelling and the bark could be peeled off of these stems rather readily.

[2] The check in each case was treated like the other samples, except that as soon as the pressure of one atmosphere was developed the stem was removed and the measurements made. Similar treatments in which no pressure was applied showed practically no movement of the dye.

the length just as would be expected from Poiseuille's formula. The following tables give the data on these tests.

Acid dyes moved readily through these tissues but basic dyes were adsorbed by the walls and scarcely entered the bark. India ink proved effective in stopping the intercellular spaces of the cortex and confining the

TABLE XVII

MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF MADRONE STEMS (*Arbutus* sp.)

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
(a) Dormant stems with bark sticking. March 15, 1930				
atm.	*min.*	*cm.*	*cm.*	*cm.*
1	1	0.10	0.30	1.50
1	2	0.50	0.75	2.50
1	4	0.50	1.50	3.00
1	8	1.00	2.00	3.00
1	12	2.50	3.00	3.00
½	4	0.40	1.00	1.50
2	4	1.00	2.00	3.00
3	4	1.00	2.50	3.00
4	3	1.00	2.50	3.00
1 check		0.10	0.20	0.50
(b) Stems with bark slipping. March 17, 1930				
1	1	0.25	0.33	1.00
1	2	0.33	0.60	2.00
1	4	0.50	1.00	2.50
1	8	0.50	1.50	3.00
1	12	1.00	2.50	3.00
½	4	0.33	0.75	2.50
2	4	0.50	1.00	3.00
3	4	1.00	2.00	3.00
4	3	1.00	2.50	3.00
1 check		0.25	0.30	0.60
(c) Stems with bark slipping. Orange G + India ink. March 17, 1930				
1	1	0.20	0.30	0.70
1	2	0.20	0.40	0.70
1	4	0.33	0.75	2.00
1	8	0.50	1.33	2.50
1	12	1.00	1.50	2.50
½	4	0.50	0.75	1.50
2	4	0.50	1.33	2.60
3	4	1.00	1.50	3.00
4	3	1.00	2.00	3.00
1 check		0.20	0.20	0.20

TABLE XVIII

MOVEMENT OF ORANGE G SOLUTION THROUGH THE BARK OF WILLOW, MARCH 14, 1930

PRESSURE	TIME	DISTANCE		
		MINIMUM	AVERAGE	MAXIMUM
atms.	*min.*	*cm.*	*cm.*	*cm.*
1	1	0.25	0.30	0.50
1	2	0.33	0.50	1.50
1	4	0.50	1.00	2.00
1	8	1.50	2.00	3.00
1	12	2.00	3.00	3.00
½	4	0.33	0.75	1.50
2	4	1.00	2.00	3.00
3	4	2.00	2.50	3.00
4	3	3.00	3.00	3.00
1 check		0.10	0.15	0.20

TABLE XIX

MOVEMENT OF ORANGE G SOLUTION + INDIA INK THROUGH THE BARK OF BARTLETT PEAR, APRICOT, EUCALYPTUS, AND REDWOOD, MARCH 19, 1930

PRESSURE	TIME	DISTANCE					
		MINIMUM		AVERAGE		MAXIMUM	
(a) Bartlett pear. Bark slipping, buds swelling							
atm.	*min.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	4	*0.50	*0.50	*0.75	*0.75	*2.00	*1.50
1	8	1.00	1.00	2.00	1.50	3.00	3.00
1	12	1.00	1.00	2.00	2.00	3.00	3.00
(b) Apricot. Bark slipping. Leaves ¼ size							
1	4	1.00	1.00	1.50	1.25	2.00	2.00
1	8	1.00	1.00	2.00	2.00	3.00	2.50
1	12	2.00	1.50	2.75	2.50	3.00	3.00
(c) Eucalyptus. Bark slipping							
1	4	1.00	0.50	1.50	1.00	2.50	2.00
1	8	1.00	1.00	1.50	1.50	2.00	2.50
1	12	1.50	1.50	2.00	1.50	3.00	1.50
(d) Redwood. Bark slipping partially							
1	6	0.33	0.50	0.75	0.75	1.50	1.50
1	12	1.00	1.00	1.50	1.33	2.00	2.50
1	18	1.00	0.50	2.00	1.50	3.00	3.00

* Duplicate experiments.

movement to the phloem. Microscopic examination showed the presence of the dye in the walls of the phloem tissues and there was no tendency for it to be localized in sieve-tubes.

Discussion

Since methods are not known at the present time by which the movement of organic materials through intact plants may be studied it is extremely difficult to prove that any particular tissue system is primarily involved in this process. The ringing experiments of CURTIS, MASON and MASKELL, and others leave little doubt but that transport of organic materials actually occurs in the bark. The sieve-tubes and the parenchyma cells with streaming protoplasm, however, both seem inadequate as conducting elements, while certain data presented in this paper indicate that the cell walls of the phloem may possibly act as the channels. From 30 to 40 per cent. of the volume of the phloem is occupied by wall material composed of about equal parts of cellulose and water. The rates calculated for flow through the walls while much too great to be explained by diffusion, seem not unreasonable in view of the possible maximum pressure gradients in the phloem which may be inferred from the exudation studies on cucurbit stems. The fact that acid dyes which move readily in the phloem walls may be forced through the bark of woody stems at rates which are similar to those calculated as necessary for storage (**21**) and growth (**48**) provides additional evidence for passage through the walls.

All of this evidence, however, is indirect and much remains to be done before any of the present theories can be accepted. In the meantime any proposed mechanism must be judged upon its general feasibility, certain characteristics being indispensable. Nourishment of the meristems and provision for growth and storage necessitate fairly rapid transport (**9**) over considerable distances. The development of tubers and flow through the trunks of tall coniferous trees are examples which present serious difficulty. The magnitude of rates in the former case, and the lack of vessels in the xylem and of sieve-pores in the phloem in the latter, practically preclude the application of previously described theories. In fact, the assumption that translocation takes place through sieve-tubes has probably hindered an unbiased study of this problem ever since these elements were named by HARTIG. Besides the resistance due to the narrowness of the pores and the fact that actual pores have not been demonstrated in many cases, the entire lack of sieve-tubes in bundle terminals of leaves and the question as to their state of differentiation in intercalary meristems are additional objections.

In considering the mechanism proposed in this paper in its relationship to the plant there are many questions yet to be settled. The state of

hydration of the phloem walls in the intact plant and the intermolecular force with which the water is held can only be surmised. It seems reasonable that the walls should occupy at least as much volume in their natural state as they do in cut sections but no actual data on this point are available.

The presence of organic solutes within the phloem walls is difficult to determine and their concentrations may only be roughly estimated. Considering the thinness of the phloem cylinder, the rigidity of the enclosing layers and the low compression coefficient of watery solutions it may be assumed that so long as the rate of flow is not too great, a relatively high concentration will be maintained throughout the phloem, the effective gradients becoming fairly short. The large surface exposed and the short distance to the xylem favor this view. This factor should considerably reduce the rates necessary especially in remote parts of the plant.

It is not essential to the proposed mechanism that there exist between "source" and "sink" a concentration gradient in the tissues as a whole. The ability of living non-photosynthesizing cells to produce a low concentration within their walls is all that is necessary. This possibly explains the lack of evidence for such gradients in much analytical data. The vertical gradients of MASON and MASKELL (**47**) probably represent the vacuolar concentrations at different levels more than they do cell wall contents, and reflect accumulation within living cells rather than conditions within the conducting tracts.

In addition to the problems already mentioned there remains the crucial one of determining the absolute resistance offered by the hydrated colloidal jell, of which the walls are composed, to mass flow of solutions. Immense amounts of internal surface are undoubtedly exposed but how this surface, composed presumably of longitudinal chains of carbohydrate molecules (**62**) reacts in comparison with the rigid walls of a glass capillary can only be surmised. The structure is less rigid than that of lignified walls, and water seems to be held less firmly than by agar or gelatin jells. The fact remains, however, that the spaces through which it is proposed that food materials in solution are passing are of molecular dimensions and irregular in form. Since no formulae are available by which to calculate pressure gradients and rates of flow through this type of material mathematical treatment is impossible. Use of Poiseuille's formula leads to high values for the required gradients and indicates discrepancies fully as great as the ones which appeared in the sieve-tube calculations. It remains for future experiments to settle this phase of the problem.

In the face of these obvious weaknesses in the proposed theory one might seem overoptimistic in attempting to view it in relation to the plant;

yet when this is done several interesting deductions become manifest. Considering the nutrition of the growing points, the exudation pressures which have been demonstrated in the phloem should force nutrients in solution into the meristems of root and shoots (**51, 52**) more effectively than root pressure (**50, 53**) or the osmotic system recently described by PRIESTLEY (**56**). In secondary roots the initiation of a cork cambium (**54**) within the phloem would be a natural result of facilitated nutrition. In storage roots, where the cork cambium surrounds a fleshy cortex, nutrition is effected through a disrupted endodermis. In primary roots an intact endodermis results in an early breakdown in the cortex. Radial arrangement in the region behind the growing root-tip allows water absorption to take place independently of the movement of foods.

The subdivision of the phloem observed to occur in storage organs such as the potato tuber, while narrowing the individual elements of this tissue, results in an increase in volume as well as in the ratio of cell wall to total phloem volume. Contrary to previous opinion (**1, 34**) this should increase its ability to conduct.

The fact that living cells (**14**) are essential to the functioning of the mechanism proposed in this work cannot be questioned. The semipermeability of the membranes separating the phloem from the adjacent tissues is directly dependent upon the nature of living protoplasm. Any condition tending to increase this permeability will allow excess leakage, and translocation will be hindered. The injection of osmotically active solutes into the phloem is dependent upon the synthetic activity of chlorophyll-bearing cells or the hydrolytic activity of storage elements. Finally the withdrawal of these substances from the channels of movement depends upon the ability of living cells to accumulate, condense, or oxidize these substances in their normal life processes.

Summary

The literature on the subject of the movement of organic materials in plants presents three main theories: (1) movement through the xylem due to a gradient of hydrostatic pressure, (2) movement through the phloem by diffusion and possibly accelerated by protoplasmic streaming, (3) mass flow through the sieve-tubes.

The first of these is not compatible with the anatomy of many plants, the second seems inadequate, the rates of diffusion and protoplasmic streaming appearing to be too slow to explain certain known rates of translocation and the third seems doubtful in view of the pressure gradients necessary to maintain flow through the constricted sieve-plate regions.

In place of these theories it is suggested that the osmotic system of PFEFFER, BLACKMAN and MÜNCH exists in the plant, the total phloem being

proposed as the channel for conduction with the majority of transport taking place in the cell walls.

In support of this proposition data from measurements and analyses on several plants are given.

Potato.—The phloem walls of the potato stolon occupy about 37 per cent. of the total phloem cross sectional area. These walls shrink to less than one-half of their original volume upon dehydration. Calculations indicate that rates of approximately one centimeter per minute through the total wall space are necessary to account for the accumulation of carbohydrates in the potato tuber.

Cucurbit.—Calculating from twenty collections of sap from pumpkin stems an average linear rate of flow of 0.81 centimeters per minute through the total phloem was recorded. Phloem walls constituted about 30 per cent. of the total phloem area in these stems. The walls shrunk 50 per cent. upon drying. Sieve-tubes occupied less than 20 per cent. of the total phloem area. Pores occupied about 8 per cent. of the sieve-plate area or 1.6 per cent. of the total phloem area. Using these values, calculations indicate that a pressure gradient of approximately 0.005 atmosphere would be necessary to force the volume of solution collected through one set of sieve-plates, or about 20 atmospheres per meter. Similar calculations show that a gradient of 6.1×10^{-5} atmosphere per centimeter would be needed to deliver this volume through a capillary tube having the same cross-sectional area as the water occupies in the phloem walls.

Further collections show that the initial rate of flow from cut cucurbit stems was about one centimeter per minute through the phloem. This rate drops off rapidly at first and then more slowly, approaching asymptotically a rate of 0.3 centimeter per minute. Calculations show that this is approximately the rate required to deliver the volume of solution necessary to form the fruits of these plants. Since rates of this magnitude cannot be accounted for on the basis of flow through the sieve-tubes it is suggested that the solution has passed through the phloem walls.

Calculations show that a rate of flow of 19 centimeters per hour would deliver enough materials through the phloem walls of Bartlett pear bark to account for the observed seasonal increase in dry weight.

A rate of 17 centimeters per hour would account for the transport of assimilate from the leaves of *Tropaeolum majus* and rates of 19.1, 15.0 and 13.5 centimeters per hour are calculated to be necessary in the phloem walls of the petiole of *Phaseolus multiflorus*.

Orange G solution was forced through the bark of several species of woody plants and rates of flow around 15 centimeters per hour, for a pressure gradient of one-third of an atmosphere per centimeter, were measured.

Acid dyes moved through the bark readily while basic ones were absorbed and failed to move.

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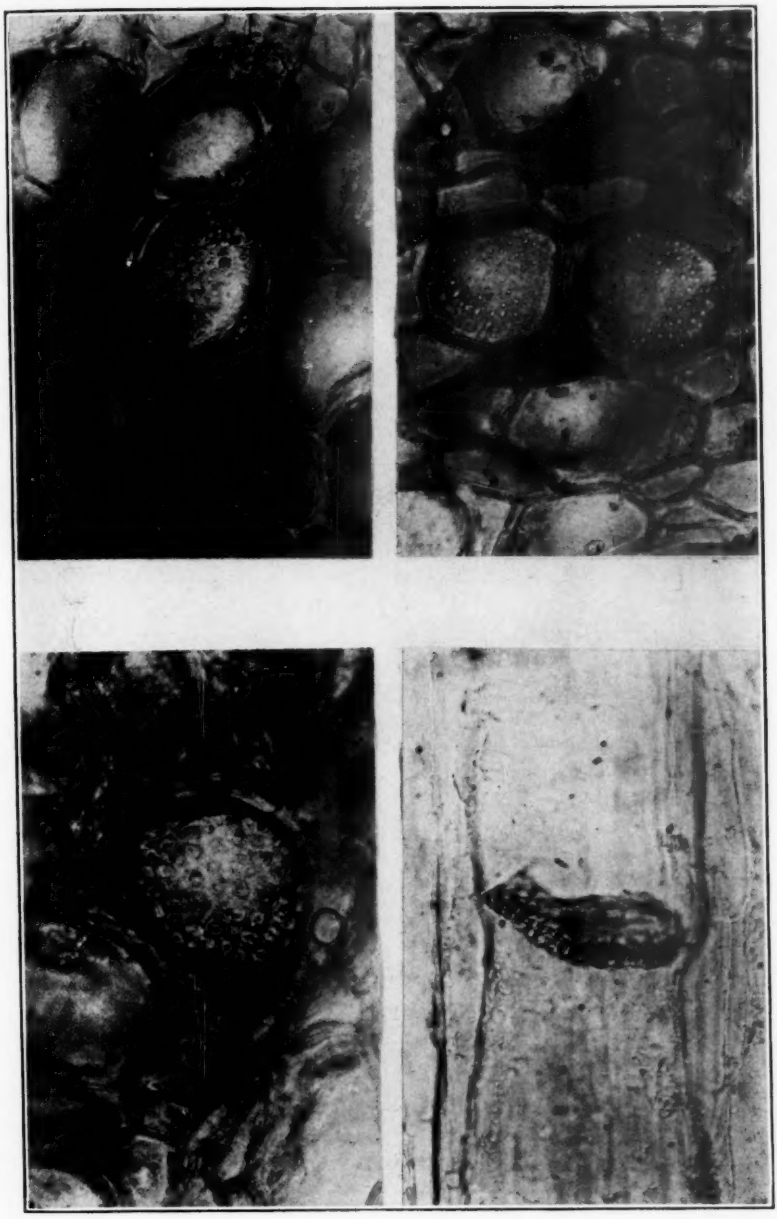
EXPLANATION OF PLATES

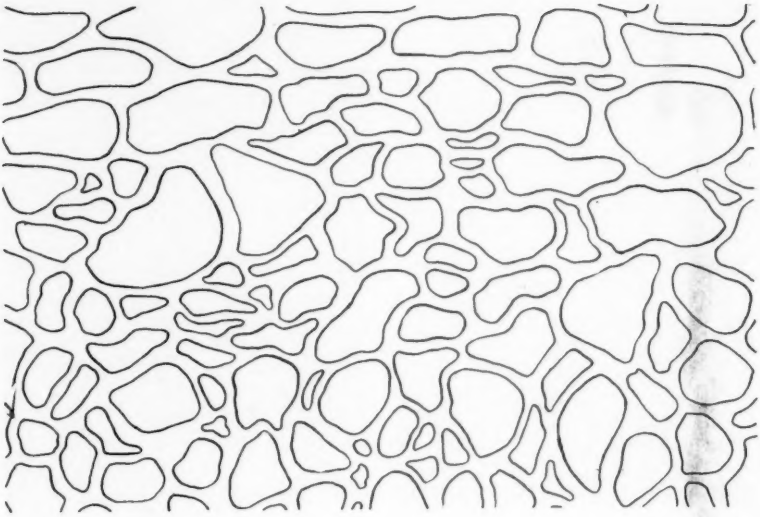
Plate II

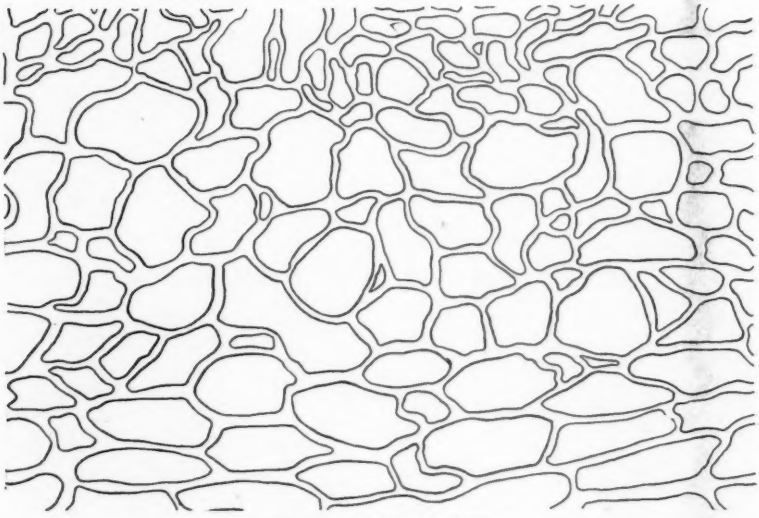
Plate II shows photomicrographs of cucurbit sieve-plates as follows:

(a) Upper left, cucurbit sieve-plate ×300.
(b) Upper right, cucurbit sieve-plates ×300.
(c) Lower left, cucurbit sieve-plate ×440.
(d) Lower right, cucurbit sieve-plate ×300.



CRAFTS—TRANSLOCATION





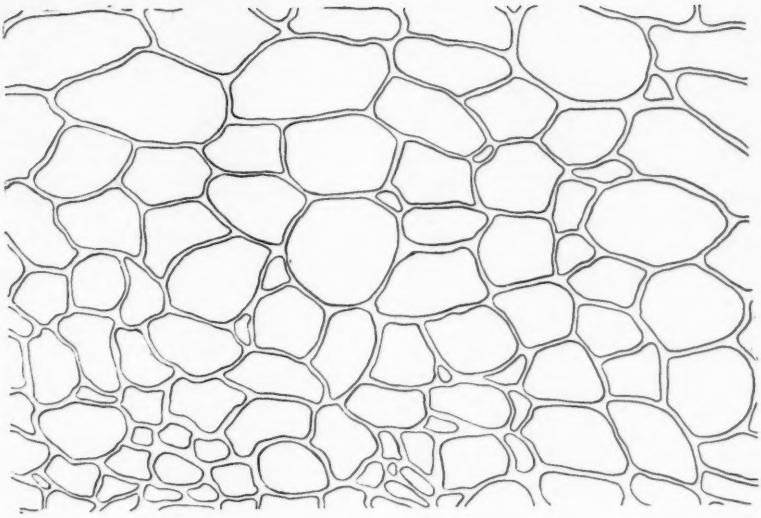
CRAFTS—TRANSLOCATION

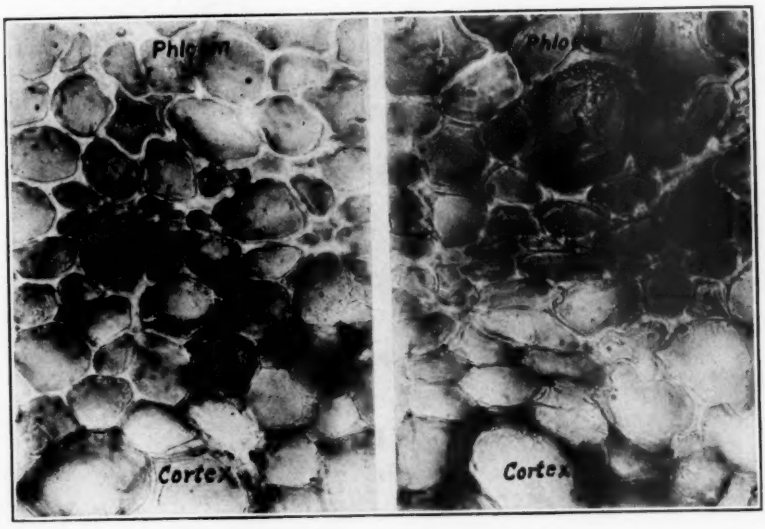
PLATE III

PLATE III shows (a) above, phloem of potato stolon ×560, fresh hand section mounted in water. Below, (b) phloem of potato stolon ×560, dehydrated in alcohol and mounted in balsam.

PLATE IV

PLATE IV shows (a) above, phloem of potato stolon $\times 560$, section air dried at 80° C. Below, (b) phloem walls of *Cucurbita pepo* $\times 265$, fresh sections mounted in glycerine.





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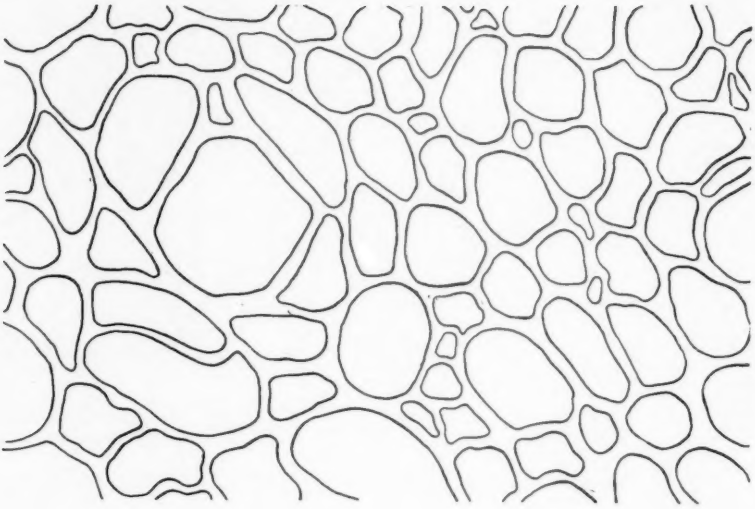

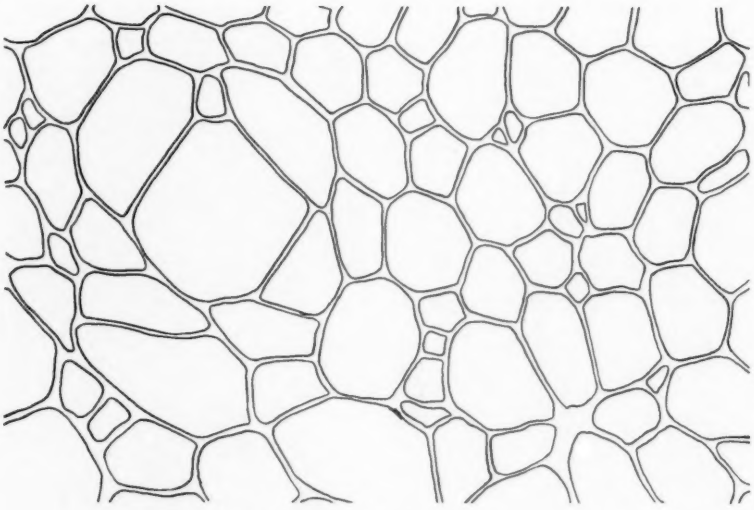

CRAFTS—TRANSLOCATION

PLATE V

PLATE V shows (a) above, phloem of pumpkin stem × 400, fresh hand section mounted in water. Below, (b) phloem of pumpkin stem × 400, same section as above, after air drying at 80° C.

42

FOOD RESERVES IN RELATION TO OTHER FACTORS LIMITING THE GROWTH OF GRASSES

L. F. GRABER[1]

(WITH FOUR FIGURES)

The immediate applied significance of the pronouncements of LIEBIG (1840) with reference to mineral nutrition of plants, not only gave rise to profound changes in soil culture and crop husbandry, but also furnished the basis of the present fertilizer industry. The classic work of SACHS, PFEFFER, KLEBS and others on the organic nutrition of plants, however, has not been utilized to full advantage. Fairly recently (1918) KRAUS and KRAYBILL (**12**) have attempted to redirect attention to such studies, and many important contributions have been made by such workers as HARVEY (**9**), MURNEEK (**14**), NIGHTINGALE (**17**), ROBERTS (**25**), JANSSEN (**11**), LEUKEL (**13**), TIEDJENS (**28**), ALBERT (**1**), NELSON (**16**), WELTON (**29**), MORRIS (**30**), and others. Unlike mineral nutrients, the carbohydrates and other organic foods cannot at present be supplied to green plants in a commercial way. They must be elaborated by the living plant itself. The plant producer can vary cultural procedures, however, so that the formation, utilization, storage and destruction of such materials may be controlled or directed and crop production be more or less precisely regulated with reference to quality and yields. Such cultural practices include defoliations by cuttings, pruning and grazing; fertilization, especially with nitrogenous fertilizers; etiolation by shading or other limitations of light; irrigation and combinations of these treatments.

Food reserves as they are discussed in this paper may be regarded in a general way as those organic compounds which are synthesized and maintained in forms capable of being subsequently utilized by the plant in the performance of its various functions. They are labile materials. They involve so great a variety of organic compounds that present chemical technique and knowledge afford a clue of identification and understanding to but relatively few of them. They represent an accumulation or surplus of materials in excess of the immediate requirements of the plant for growth and maintenance. Their utilization is, essentially, most abundant in an environment favorable for growth extension and in those meristematic regions of the plant where the metabolism is most active. Generally, their storage occurs during periods when the accretions in dry weight are most rapid.

[1] Contribution from the Department of Agronomy of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin. Published with the approval of the director.

With the progressive enlargement of the photosynthetic area in plants, sufficient new foods are manufactured not only to provide for growth extensions but also to provide for accumulations and storage of such foods for subsequent growth. However, when photosynthesis is interrupted by frequent and complete removals of the green parts, the plant may not have an opportunity to replenish the losses of previously stored foods which were used up in its regenerative activity. Especially is this true when regeneration of new growth is stimulated by mineral nutrients, moisture and temperature, and when photosynthesis is further curtailed by shading or other forms of light deficiency. Under such circumstances the growth rate soon declines and although the levels of reserves might be sufficient to maintain the existence of the plant for some time, death eventually ensues.

The data presented in this paper, represent an attempt to correlate certain cultural procedures with the utilization and accumulation of organic foods. There is a general recognition of the ready response of plants to fertilization, moisture and to changes in temperature and light conditions, but when cultural practices involve defoliation, the responses are usually delayed and are often ascribed to some other cause even though the effect on the internal environment may be pronounced and be clearly reflected in subsequent growth. Thus alfalfa (*Medicago sativa* L.) with an abundance of stored foods may yield more during one season of growth if cut frequently rather than infrequently, but the ill-effects of frequent cutting are usually pronounced the following season. Similarly, blue grass (*Poa pratensis* L.) may be grazed closely and early for a season or more with good results, but the decline in its productivity is certain, even though not readily recognized because of wide variations in seasonal conditions, and because of the growth of other plants among the blue grass, which have not been affected so seriously by the grazing treatment. Food reserves are so intimately associated and inter-related with all other limiting factors of growth that it is often difficult to differentiate from drought, winter injury, infertility, or some other factor or factors, the particular effect a lack of such reserves may have played in developing any specific condition under consideration.

Reserves in relation to morphology and growth habits

The wide variations in the responses of agronomic plants to specific practices of top growth removal are correlated with some of their general characteristics of growth such as the duration of the succulent period and the structure and arrangement of the photosynthetic parts. PIERRE and BERTRAM (**23**) have studied the storage of organic foods as they affect the tops and roots of kudzu (*Pueraria thunbergiana*), a leguminous vine which is being utilized for forage in southern United States. Accord-

ing to PIPER (**24**) this is a very late blooming plant with a long period of succulent growth, maturing only occasionally in the latitude of Washington, D. C. PIERRE and BERTRAM report that, in Alabama, "the reserve starch and nitrogen were found to be less than half as much in the roots of plants receiving six cuttings per season for two years as in roots of plants receiving four or a less number of cuttings." During a period of two years, the weight of the roots of kudzu increased 1250 per cent. when the top growth was cut but once annually near maturity, while with six cuttings of immature top growth the roots actually decreased in weight. In the third year, the yield of top growth from the plants cut six times was only about one thirty-third of that obtained from plants cut but once annually. Other cutting treatments were tried but these will suffice to illustrate the marked responses of the kudzu plant to cultural treatments which affect the food reserves. With a relatively long period of succulence and a rapid rate of growth, several cuttings during the growing season apparently give little opportunity for sufficient accumulation of stored foods to assure the maintenance of a desirable productivity in this perennial.

The alfalfa plant has a high metabolic activity, with great ability for regeneration; and its reserves are rapidly reduced by frequent cuttings. It is obvious, on the other hand, that the reserves of small short plants such as blue grass (*Poa*) or creeping bent grasses (*Agrostis*) would be much less affected by frequent mowing with a field mower or a lawn mower, partly because of the slow recovery in vegetative extension, and particularly because of the greater proportion of the photosynthetic area remaining after each cutting. Such green remnants of herbaceous plants have the capacity to replenish, in part, the losses of reserves sustained by frequent cuttings, close grazing or other forms of removal. Under field conditions a much greater proportion of the photosynthetic area of tall, erect plants is removed by cutting or by close grazing than is true of plants whose leaves diverge largely from short branches of rhizomes or stolons, or of plants with short stems and a decumbent or rosette type of growth where the photosynthetic area consists largely of basal leaves near the soil surface. The general structure of the plant, therefore, has an important bearing on its capacity to maintain sufficient reserves for its survival under exacting grazing or cutting treatments. The persistence of blue grass (*Poa*), plantain (*Plantago*), dandelions (*Taraxacum*), purslane (*Portulaca*), creeping spurge (*Euphorbia*), and many other prostrate plants which maintain themselves in fields, lawns and pastures is thus explained in part. As shown by GRABER, NELSON, LEUKEL and ALBERT (**8**) twenty-two close cuttings with a lawn mower did not kill blue grass (*Poa pratensis*) though it

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The alfalfa plant has a high metabolic activity, with great ability for regeneration; and its reserves are rapidly reduced by frequent cuttings. It is obvious, on the other hand, that the reserves of small short plants such as blue grass (*Poa*) or creeping bent grasses (*Agrostis*) would be much less affected by frequent mowing with a field mower or a lawn mower, partly because of the slow recovery in vegetative extension, and particularly because of the greater proportion of the photosynthetic area remaining after each cutting. Such green remnants of herbaceous plants have the capacity to replenish, in part, the losses of reserves sustained by frequent cuttings, close grazing or other forms of removal. Under field conditions a much greater proportion of the photosynthetic area of tall, erect plants is removed by cutting or by close grazing than is true of plants whose leaves diverge largely from short branches of rhizomes or stolons, or of plants with short stems and a decumbent or rosette type of growth where the photosynthetic area consists largely of basal leaves near the soil surface. The general structure of the plant, therefore, has an important bearing on its capacity to maintain sufficient reserves for its survival under exacting grazing or cutting treatments. The persistence of blue grass (*Poa*), plantain (*Plantago*), dandelions (*Taraxacum*), purslane (*Portulaca*), creeping spurge (*Euphorbia*), and many other prostrate plants which maintain themselves in fields, lawns and pastures is thus explained in part. As shown by GRABER, NELSON, LEUKEL and ALBERT (**8**) twenty-two close cuttings with a lawn mower did not kill blue grass (*Poa pratensis*) though it

lessened its productivity, while only nine such cuttings resulted in death to nearly all the plants of alfalfa. Close cutting of blue grass with a lawn mower reduced subsequent productivity much more than "tall" cutting with the same implement.

In experiments conducted by the writer (**7**) during 1929 with blue grass seeded in 1928, the simple expedient of setting the lawn mower to cut "high" produced a thick turf free of weeds in contrast to one which was thin and weedy due to close cutting with the same frequency. An uncut remnant of one and one-half inches of the foliar parts of grasses is decidedly more effective in maintaining a satisfactory level of reserves than a half inch of such growth. This is not only significant in the maintenance of blue grass lawns where density of growth and freedom of weeds are factors which add to the value of the turf, but it has applications in the management of pastures. ALDOUS (**2**) indicates that the unfavorable effects of frequent clipping of prairie grasses are partially eliminated by increasing the height of clipping. STAPLEDON and MILTON (**26**) report that cocksfoot (*Dactylis glomerata*) plants cut frequently to a six inch level have considerably outyielded those cut to ground level. The development of tillers and roots was also much greater under the less drastic system of cutting.

The Hohenheim system

Recently, investigations have been started in America to determine the merits of the Hohenheim system of pasture management. In the main, this German system involves heavy fertilization (with mineral and especially nitrogenous fertilizers) of permanent pastures which are grazed closely at intervals throughout the year. It is evident that the success of this system of rotational grazing must make provision for the maintenance of a sufficient surplus of carbohydrates. With the stimulus of a liberal supply of nitrogen, rapid growth not only consumes organic foods for energy and cell wall materials, but the carbohydrates are utilized in the synthesis of large amounts of proteins. Heavy nitrogenous fertilization of blue grass also makes possible the stimulation of top growth at the expense of root growth, especially where grazing or cutting is sufficiently delayed to produce etiolation effects in the buds and meristematic regions of the leaves. However, by avoiding extremely early and close spring grazing and by proper extension of the intervals between grazing, the maintenance of an adequate carbohydrate reserve is readily made possible. The agronomic importance of ascertaining the responses of pasture grasses to varying "periods of rest" in rotational grazing is very evident especially under conditions of a very favorable soil and climatic environment.

While judicious fertilization may for a time stimulate the utilization of carbohydrate reserves, it is not amiss to appreciate that such stimulation

may also accelerate their accumulation when the plant is given an opportunity for such storage. Fertilization often trebles the productive capacity of blue grass and other pasture grasses grown on soils deficient in general fertility. Such enhanced potentialities in the synthesis of foods must greatly aid the green remnants which escape grazing to maintain a productive level of reserve foods in such grasses. It is primarily when these remnants are kept sufficiently reduced by close grazing or cutting treatments that the stimulation in growth from judicious fertilization is apt to hasten the decline of the reserves to a degree of serious curtailment of subsequent productivity.

Survival values and reserves

Grasses in lawns, golf courses, and pastures may exist for many years even though they receive treatments which are not conducive to the maintenance of a high reserve content. It is always to be recognized that plants with low supplies of organic foods may survive even though their capacity for survival is greatly diminished. In extreme cases, however, the maintenance of special external conditions is necessary for such survival and the constant watering and careful fertilization of golf greens and closely cut blue grass lawns are examples of such effort. Such special conditions augment the limited root growth of low reserve plants and aid the synthesizing areas which escape removal, in maintaining a level of reserve foods sufficient for the survival of the plants. Under field conditions, however, the limitations of root growth, and the modifications of the internal environment resulting from low reserves may reduce the absorptive capacity of a plant so greatly and may so increase its susceptibility to drought, winter injury, weed encroachments, insect injury and other hazards as to jeopardize its permanence. Such secondary effects often intensify the influence of low reserves on the productivity of plants, and since they appear so obvious, they may cloak this primary cause of many forms of deterioration in economic plants.

Observations made by the writer (**3**) in southwestern Wisconsin, where permanent blue grass grazing lands have been grazed for thirty years or more, have revealed that insect injury from white grubs (*Phyllophaga* spp.) and weed infestations appeared much more abundant in pastures which had been prematurely over-grazed or which were deficient in fertility or where soils were thin and dry due to outcroppings of limestone or flint, or where combinations of such conditions occurred. While these factors may encourage insect infestations, they also limit the amount of subterranean growth, and, in general, where such growth was least, insect injury was most abundant. The survival of economic plants, their productivity and their competitive efficiency under field conditions are dependent upon

many inter-related factors but organic reserves are among the most important.

The experiments reported in this paper show some of the correlations between agronomic practices and food reserves as they affect productivity under various soil environments and as they affect the ecological relationships of such plants under field conditions. KRAUS and KRAYBILL state that "a plant at any particular time represents the result of all the environmental forces acting upon it, and it is either in a state of equilibrium with such forces or in a state of becoming so adjusted." The adjustment of plants to those conditions which are brought about by such cultural treatments as grazing, cutting, fertilization and irrigation, is highly complex and must be approached from a study of both internal and external relations.

Cutting treatments in relation to productivity of blue grass with and without fertilization

EXPERIMENT I

A strip of land on the University Farm, Madison, Wisconsin, classified as Miami silt loam, was summer fallowed during 1925, and up to July 22, 1926, on which date it was sown with blue grass seed (*Poa pratensis* L.) at the rate of 100 pounds per acre. By fall a thick uniform growth of grass had developed to a height of about four inches. No flower stalks appeared that season nor was the grass cut. On April 11, 1927, the grass was rolled twice with a three-ton roller so as to produce a smooth surface for uniform mowing with a lawn or field mower. The area was divided into four plats of 1/35 of an acre, each designated by the letters A, B, C, and D. Plats A and C were not fertilized during the period of the experiment, but plats B and D received the following applications expressed in pounds per acre:

March 18, 1927—220 pounds of super-phosphate (16 per cent. P_2O_5)
140 pounds of potassium sulphate (40 per cent. K.)
70 pounds of sodium nitrate (16 per cent. N.)
May 26, 1927—140 pounds of ammonium sulphate (20 per cent. N.)
July 30, 1927—200 pounds of ammonium sulphate (20 per cent. N.)
May 15, 1928—200 pounds of ammonium sulphate (20 per cent. N.)
July 12, 1928—350 pounds of ammonium sulphate (20 per cent. N.)

Thus, a total of 79 pounds of elemental nitrogen per acre was applied in 1927 and 110 pounds in 1928. The soil was naturally abundant in lime and in a fairly high state of fertility. The summer fallowing which preceded seeding probably increased the available fertility very considerably.

In 1927, two different cutting treatments were employed on the fertilized and unfertilized areas. Plats C (unfertilized) and D (fertilized) were cut six times with a lawn mower (set to mow about one inch above the

surface of the soil) on April 6, 29, May 11, 27, June 8 and 24, 1927. Plats A (unfertilized) and B (fertilized) were cut only once with a field mower when near maturity on June 24, 1927.

In 1928, the plats or representative portions of them were cut twice; once at maturity on July 7, 1928, and again on August 31, 1928. All cuttings were weighed in the green state and a representative sample of from 500 to 1000 grams was taken for a determination of moisture content so as to arrive at the dry weights on the basis of oven-dried grass. No attempt was made to accomplish complete desiccation although the samples were kept in a drying room for periods of from four to six days at temperatures ranging from 100° F. to 110° F. which, it is believed, provided dry-

TABLE I

COMPARATIVE AMOUNTS OF BLUE GRASS (*Poa pratensis* L.) OBTAINED FROM SIX CUTTINGS AT IMMATURE STAGES OF GROWTH AND ONE CUTTING NEAR MATURITY WHEN GROWN ON FERTILIZED AND UNFERTILIZED SOIL. BLUE GRASS SOWN JULY 22, 1926. RESULTS EXPRESSED IN POUNDS OF OVEN-DRIED GRASS PER ACRE

PLOT	FERTILIZATION	DATES AND AMOUNTS OF OVEN-DRIED TOP GROWTH REMOVED DURING 1927						
		4/6	4/29	5/11	5/27	6/8	6/24	TOTAL
C	None	244	255	332	156	133	149	1269
A	None						2930	2930
D	Abundant	146	203	342	183	236	266	1376
B	Abundant						3563	3563

TABLE II

THE INFLUENCE OF CUTTING TREATMENTS IN 1927 ON THE PRODUCTION OF BLUE GRASS DURING 1928 EXPRESSED IN POUNDS PER ACRE

PLOT	FERTILIZATION	DATES AND AMOUNTS OF OVEN-DRIED TOP GROWTH REMOVED DURING 1928				
		7/7/28			8/31/28	TOTAL FOR 1928
		LEAF GROWTH	FLOWER* STALKS	TOTAL	LEAF GROWTH ONLY	
C	None	448	152	600	603	1203
A	None	1443	279	1722	1037	2759
D	Abundant	1609	353	1962	2674	4636
B	Abundant	2630	523	3153	3315	6468

* Flower stalks include leaves diverging at nodes.

ing to a fairly uniform state. All yields were calculated on the basis of such oven-dried hay, and are given in tables I and II.

RESULTS.—The abundant rainfall, measuring 12.41 inches, during April, May and the first 24 days of June, 1927, benefited the growth of the blue grass as did also the favorable conditions of the soil on which it grew. However, when the cumulative effects of photosynthesis were diminished by frequent cuttings, the production of dry matter during a given period was

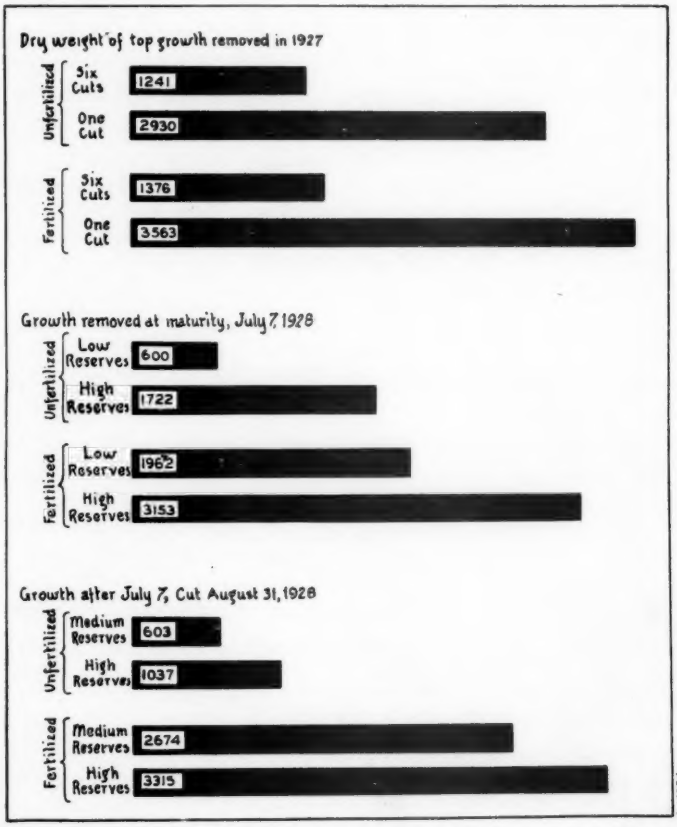

FIG. 1. Productivity of blue grass with relation to food reserves and fertilization. Six cuttings of blue grass made with a lawn mower in 1927 on April 6, 29, May 11, 27, June 8 and 24, not only gave less than half the amount of dry matter obtained with one cutting taken near maturity on June 24, 1927, but greatly reduced the productivity of the blue grass the following year as measured on July 7 and August 31, 1928. Amount of top growth removed is expressed graphically on the basis of pounds of oven-dried grass per acre.

greatly reduced. Thus, on the unfertilized soil, six cuttings of immature blue grass (figure 1) yielded but 43 per cent. of the amount of dry matter obtained from one cutting near maturity (on June 24) while with fertilization, six cuttings produced only 38 per cent. of the amount of dry matter obtained with one cutting.

The productive responses of blue grass to the fertilizations made on March 18 and May 26, 1927, were much less pronounced when six cuttings were taken during the spring and early summer period (up to and including June 24, 1927) than when one cutting was taken near maturity on June 24. Comparing the amount of growth removed from the fertilized and unfertilized plats of blue grass given identical cutting treatments, fertilization increased the yield (table I) of blue grass cut six times, 11 per cent. and that cut once, 21 per cent.

During the last six days of June and in July and August, the rainfall was only 2.43 inches so that little growth occurred during this period but in September and October when 10.45 inches of rain fell the blue grass made considerable recovery. This growth was not removed, which gave excellent opportunity for fall storage of organic foods. However, the six early cuttings prior to June 25, 1927, had a profound effect on the productivity of the blue grass in 1928. This is shown by data in table II and graphically by figure 1.

Plats B and D were fertilized again with ammonium sulphate in 1928 on May 15 and July 12, with applications sufficient to apply 110 pounds of total elemental nitrogen per acre, while plats A and C remained unfertilized. All plats were cut when fully mature on July 7, and again on the last day of August. This made possible a measurement in 1928 of the residual effects of the cutting treatments applied in 1927 on the productivity of the grass grown on the fertilized and unfertilized soil.

Without fertilization the blue grass with "high" reserves produced on July 7, 1928, nearly three times the amount of dry matter (figure 1) obtained from blue grass with "low" reserves. With fertilization the yields of blue grass with "high" reserves were 1.6 times those obtained from the blue grass "low" in reserves. Low reserves imply limited root growth, limited absorptive capacity and limited drought resistance, which conditions may well have been obviated, in part, by heavy fertilization. The designations "low" and "high" with reference to reserves are based only on previous cutting treatments and not on chemical analysis. These comparative terms are used to simplify the discussion.

It is not assumed that the deficiencies in reserves which were occasioned by the six cuttings in 1927 were alone responsible for the reduced yields on July 7, 1928, but in all probability, they were of major importance. Cutting treatments which reduce food reserves tend to retard subterranean

growth and may well lower the absorptive capacity of the plants. The marked responses from fertilization in 1928, resulting in increases of dry matter from 234 to 385 per cent., offer a striking contrast with increases of only 11 to 21 per cent. in 1927. In part, at least, such increases indicate changes in available fertility (particularly nitrogen) and losses of nutrients from the removals of grass in 1927.

The abundant rainfall during the latter part of June and in July and August, 1928, made possible another measurement of the productivity of blue grass on August 31, 1928. The fact, however, that the blue grass "low" in reserves was allowed to mature before cutting in 1928 made possible some re-establishment in the supplies of stored foods of the blue grass cut six times in 1927. For this reason, the designation, in figure 1, for the growth of blue grass after July 7, was changed from "low reserves" to "medium reserves" to approximate the probable situation of the organic foods in relation to the yields of blue grass determined on August 31. The growth removed at this time was all leaves, no flowering stalks having developed after cutting on July 7. While the grass with high reserves again yielded most, whether grown on fertilized or unfertilized soil, the differences in yields with reference to reserves are not as pronounced on August 31, 1928, as they were on July 7, 1928. Allowing blue grass with "low" reserves to fully mature apparently resulted in sufficient recuperation of the organic food surplus to increase the general metabolism and subsequent summer productivity. Whatever this may have involved in the way of additional root and rhizome development or in a greater absorptive capacity of the subterranean parts or in a greater release of energy for more rapid growth, such conditions were not sufficient to restore the full productivity of the plants as expressed by the blue grass with "high" reserves.

Reserve foods and fertilization.—The yields illustrated in figure 1 are indicative of the fact that although the soil may be abundantly supplied with available nitrogen, phosphorus, potash and all other requisites of growth, the plant may be deficient in organic materials for the greatest expression of its productive capacity. The productivity of blue grass with relation to nitrogenous fertilization is dependent, not only, upon adequate supplies of moisture, phosphorus, potash and other limiting elements within the soil, but upon the reserve foods within the plant which are also limiting factors of growth. A relatively greater response from nitrogenous fertilization occurred with blue grass having low reserves than with similar fertilization of blue grass where the reserves had not been lowered by previous cutting treatments. Fertilization during 1927 and 1928 increased the yields (taken on July 7, 1928) of blue grass with low reserves 327 per cent. in comparison with only 183 per cent. increase from the same fertiliza-

tion of blue grass with high reserves. On August 31, 1928, the increase from fertilization was 443 per cent. for the grass then having medium reserves compared with 320 per cent. for the grass with high reserves. That an explanation of such variations in the responses of high and low reserve grass to similar fertilization may rest, in part, on a greater capacity for the absorption and utilization of limited supplies of soil nutrients by plants having abundant food reserves is indicated by the recent work of STOKES, LEUKEL and BARNETTE (**27**). They found in the crowns of Napier grass (*Pennisetum purpureum*) which had been irrigated for three years with sewage effluent, that the quantities of dry matter, various carbohydrate compounds, total available carbohydrates and total nitrogen were approximately three and one-half times that of similar materials in the crowns of non-irrigated plants. The percentages of these organic compounds were quite similar, however, in the plants with and without irrigation. "It is apparent," they state, "that the continued large yields of Napier grass silage from the irrigated plats after the discontinuation of the irrigation with sewage effluent, cannot be explained alone on the basis of a greatly increased soil fertility of the irrigated plats nor to water alone as shown by the yields of the city water irrigated plat." The production of a larger plant system through irrigation with sewage effluent increased the elaboration of organic foods, a condition "reflected in the increased growth of the lower storage parts of the plants." Analysis of crowns subsequent to the discontinuance of irrigation, indicated that with a more extensive absorptive system and with more abundant carbohydrate reserves, a greater efficiency in the utilization of plant nutrients (particularly nitrogen) occurred.

Growth recovery.—The growth recovery of the blue grass on the fertilized plats after cutting on July 7, 1928, was much more rapid and uniform at the outset with the grass having been cut six times in 1927 than with that cut but once in 1927. During the latter part of July, 1928, the former plats of blue grass were uniformly green while the latter still appeared brown and somewhat patchy, the new growth not being thick enough to hide uniformly the dead leaves of the old stubble. On the unfertilized plats a somewhat similar contrast was apparent but was much less pronounced. It is well known that thick stands of the second year's growth of sweet clover (*Melilotus alba*) often fail to recover after late cutting. Here the young shoots and axillary buds at the lower nodes of the stems have been seriously injured by etiolation from the tall dense growth above but when this legume is grazed or clipped at immature stages, sunlight is provided for the basal leaves and buds and they continue to develop.

In this experiment, the growth of blue grass with high reserves and high fertility was so rapid and so abundant during early summer that the devel-

opment of the buds on the rhizomes or leaves diverging from the upright branches of the rhizomes was largely inhibited by the competition of the dense growth above. The basal portions of the older leaves were largely killed by the effects of etiolation and rather sudden light exposure after cutting. These factors involved delay in recovery because subsequent regeneration occurred largely from dormant buds on the rhizomes or the uninjured leaves of their branches. With less abundant top growth during spring and early summer, etiolation occurred to a much less extent and the new leaf growth was able to elongate in competition with the older growth so that when the latter was removed the leaves which partly or completely escaped mowing were in a state of growth and continued to develop after cutting.

When comparative yields on August 31, 1928, are considered, it is apparent that in this experiment, the productive capacity of blue grass well supplied with reserve foods and with abundant fertility was sufficient to compensate for the losses sustained by slow recovery resulting from the retarding effects of previous heavy growth. Such delayed recovery would, of course, prove very disadvantageous in the maintenance of lawns and of other turfs where uniformity is desired and it is a factor of importance with pasture management where heavy nitrogenous fertilization is practiced. When cutting is sufficiently delayed beyond maturity the desiccation of the old growth may so reduce its competitive effects that the development of new buds and leaves occurs and recovery after cutting is more rapid.

Ecological considerations.—The amounts of foreign growth in the plats during 1927 was very small. Just before cutting the blue grass on August 31, 1928, the weed growth was considerable, and it was removed from part of each plat (1/70 acre) with each species being counted as summarized in table III. On the unfertilized soil, the weeds were ten times more abundant in the blue grass cut six times in 1927 than were cut once that year. Fertilization, however, was a very potent factor in preventing the ingress of foreign plants. The weeds in the fertilized blue grass cut six times in the previous year were only about one-seventh of the number found in similar blue grass growing on unfertilized plats. The failure of the fertilized plats of blue grass with "high" reserves to recover rapidly gave purslane and barnyard grass a vigorous start before the grass recovered sufficiently to become a competitive factor. Both of these weeds in absence of initial competition were favored by fertilization and abundant rainfall which occurred during the summer of 1928. Purslane was present only in the fertilized plats of "high" reserve blue grass where delay in growth recovery occurred after cutting on July 7, 1928. Barnyard grass and pigweed were most abundant in these plats while other weeds were absent or

TABLE III

NUMBER OF WEEDS IN PLATS OF BLUE GRASS OF ONE-SEVENTIETH OF AN ACRE AS AFFECTED BY FERTILITY, CUTTING TREATMENTS AND ORGANIC FOOD RESERVES. DETERMINATIONS MADE ON AUGUST 30, 1928

SPECIES	SIX CUTTINGS 1927 ONE CUTTING JULY 7, 1928		ONE CUTTING JUNE 24, 1927 ONE CUTTING JULY 7, 1928	
	FERTILIZED	UNFERTILIZED	FERTILIZED	UNFERTILIZED
Witch grass (*Panicum capillare* L.)	8	535	26	8
Foxtail (*Setaria glauca* L.) (*Setaria viridis*)	48	260	6	44
Purslane (*Portulaca oleracea*)	0	0	280	0
Barnyard grass (*Panicum crusgalli* L.)	15	11	158	10
Horseweed (*Erigeron canadensis* L.)	0	234	0	22
Others	92	83	20	45
Total	163	1123	490	129

sparse. Foxtail (*Setaria*), witch grass (*Panicum*), dandelions (*Taraxacum*), cinquefoil (*Potentilla*), horseweed (*Erigeron*), and field sorrel (*Oxalis*) were conspicuous in the unfertilized plats of the blue grass given six cuttings in 1927.

While an invasion of weeds occurred in some of the plats during the summer of 1928, the only foreign growth which was prevalent in 1929 was white clover (*Trifolium repens*). Neither weeds nor clover appeared in any of the plats heavily fertilized with nitrogen, but in the unfertilized plats especially of the blue grass cut frequently in 1927 white clover was very abundant. The generous rainfall during the summer of 1928 made it possible for the dormant seeds in the soil to establish seedling plants of white clover which, in turn, were protected by an abundance of snow during the following winter. The soil was favorable for growth of this legume so that the external environment made possible its sudden appearance in the plats of blue grass which had not been fertilized.

The prevalence of plants other than blue grass in the plats of this experiment is somewhat analogous to the general situation observed in the pasture lands of the blue grass region of southwestern Wisconsin. The weed population of grazed blue grass may vary widely in kind from blue grass given various cutting treatments, but there are some noteworthy similarities. In general, it can be said that those factors which encourage the growth of blue grass tend to retard the ingress of weeds or clovers in

such blue grass. Thus, in the pastures of southwestern Wisconsin both weeds and white clover are generally very sparse where soils are fertile and retentive of moisture, and where the blue grass is grazed judiciously and is well supplied with reserve foods. See fig. 2 for influence of grazing on the flora of blue grass pastures. This condition tends to maintain a heavy

FIG. 2. Grazing practices influence flora of blue grass pastures. Close, early spring grazing has lowered the reserves and the competitive efficiency of the blue grass on the hillside (to right) and a heavy growth of unpalatable ragweeds (*Ambrosia*) has occurred in the pasture while with deferred and lighter grazing (left) these weeds are absent. The portions of the pastures shown above have never been plowed and although hilly the soil is regarded as being in a fairly fertile condition.

sod and during the spring season, at least, an abundant top growth, both of which are effective in preventing the entrance of foreign growths. It is not meant to imply that blue grass with optimum conditions for growth is able to exclude all forms of weed growth, but rather to indicate that when the nitrates and other mineral elements of the soil, and the organic reserves of grasses in grazed pastures are maintained in an optimum state, the competition of the blue grass is usually sufficient to prevent or retard the ingress of many undesirable plants as well as desirable ones.

The requirements of white clover for lime, phosphate, and potash are pronounced and when these nutrients are deficient in soils of grasslands, this clover is usually sparse and weeds are often abundant. The preva-

lence of this legume in southwestern Wisconsin is also influenced by climatic conditions of both winter and summer. It is probably less sensitive to drought and surely much more susceptible to winter injury than blue grass.

Many weeds such as ragweed (*Ambrosia*), dandelion (*Taraxacum*), vervain (*Verbena*), thistle (*Cirsium*), yarrow (*Achillea*), and others have a distinct advantage in competing with early and closely grazed blue grass. Such weeds may prevail because of their drought resistance or because of their general structure or inedibility or unpalatability or combinations of these characteristics which permit a partial escape from grazing even when pastures are heavily grazed. They have the advantage of being able to provide for permanence by forming seed and by maintaining reserves of organic foods. Tall plants which are palatable and with foliar parts located so as to be readily grazed, often disappear or fail to become established because with constant grazing they may not be able to form seed or maintain an adequate supply of reserve foods for their survival.

When grasses are permitted to make considerable growth before cutting or grazing, they offer greater resistance to the establishment of seedlings of foreign plants than if cut frequently. As shown by GRABER (**4, 5, 6**) viable seeds of legumes sown on dense sods or where accumulations of old grass had occurred, failed to establish plants; but when such old grass was burned off, or when the sod was scarified so as to provide soil contacts for the viable seeds, the seedings were successful, if soil, climatic and other conditions were adapted to their requirements for growth. The opportunity for plants to establish themselves by seed dispersal in permanent grasslands is dependent upon factors which influence the possibilities of soil contacts for the seed as well as an environment sufficiently favorable for subsequent development. Such possibilities are often enhanced by the frequent removals of accumulations of top growth by close grazing or cutting.

There are, of course, many other external factors, such as climatic conditions, the prevalence of insects which feed on subterranean growth, the acidity of the soil, its content of nitrogen (see fig. 3) and other mineral components, its moisture holding capacity and its drainage, all of which affect the flora of permanent grasslands; but the factors which affect the internal environment of pasture grasses are also important. Under the complexities of field situations organic food reserves of blue grass as affected by cultural treatments are not only correlated with other factors which affect the quantitative responses of this plant, but such surplus foods also have an ecological significance in maintaining a high survival value and a competitive efficiency when the growth of this important grass is jeopardized by climatic, insect and weed hazards.

FIG. 3. Nitrogenous fertilization curtailed growth of white clover and weeds in blue grass. A volunteer growth of white clover occurred only in the unfertilized blue grass (left) while its absence in the adjacent plat resulted from abundant applications of nitrogenous fertilizers. Photo taken June 25, 1929.

Nitrogen relations

EXPERIMENT II

On July 14, 1925, blue grass (*Poa pratensis* L.), red top (*Agrostis alba* L.), fescue (*Festuca rubra fallax*), and timothy (*Phleum pratense*) were each sown separately in four plats of one twenty-fifth of an acre each of a Miami silt loam soil which had been summer fallowed for two years. A thick dense growth of these grasses resulted that year, and they attained a height of from three to five inches in the fall of 1925. No cuttings of top growth were made in 1925. Beginning in 1926 one-half the area of each plat was top dressed with applications of fertilizer on the dates and in the amounts per acre as follows:

April 19, 1926—500 pounds of 4–16–6 fertilizer (4 per cent. N, 16 per cent. P_2O_5, 6 per cent. K_2O).
July 13, 1926— 500 pounds of 4–16–6 fertilizer.
3000 pounds of dolomitic limestone.
March 18, 1927—125 pounds of ammonium phosphate (10.63 per cent. N).
May 20, 1927—125 pounds of calcium cyanamid (21.73 per cent. N).
July 30, 1927—200 pounds of ammonium sulphate (20 per cent. N).
May 12, 1928—125 pounds of ammonium sulphate (20 per cent. N).

The fertilized and unfertilized half of each plat was divided into two equal parts of 1/100 of an acre each and a plat of each grass on the fertilized area and one on the unfertilized area was cut once with a field mower when at or near maturity on July 10, 1926. The remaining two plats of each grass were cut (with a mower) on May 22, June 12, July 10, and August 18, 1926. The yields of the grasses with these cutting treatments are given in table IV.

In 1927, the plats of grass cut four times in 1926 were given a more intensive cutting treatment by close mowings (about one inch above soil surface) with a lawn mower on April 6, May 2, 21, 30, June 8, 24, and October 28. The plats of grass cut only once in 1926 were again cut only once on June 24, 1927.

In 1928, all plats were cut at or near maturity on July 7 to measure the after effects of previous cutting treatments.

TABLE IV

NITROGEN REMOVED WITH GRASSES GIVEN FREQUENT AND INFREQUENT CUTTINGS OF TOP GROWTH. GRASSES SOWN JULY 14, 1925

GRASS	FERTILIZATION	POUNDS PER ACRE OF OVEN-DRIED GRASS AND OF ELEMENTAL NITROGEN IN GRASS								
		1926			1927			1928		
		No. CUTS	YIELD	N	No. CUTS	YIELD	N	No. CUTS	YIELD	N
Blue grass (*Poa pratensis* L.)	None	1	5050	58	1	4656	55	1	1133	13
		4	6969	199	7	2093	57	1	764	12
	Abundant	1	4867	72	1	6424	80	1	4369	68
		4	7773	193	7	3571	111	1	2804	61
Fescue (*Festuca rubra fallax*)	None	1	5916	101	1	2322	20	1	888	8
		4	5316	140	7	1559	35	1	978	7
	Abundant	1	5895	77	1	3335	45	1	3021	34
		4	6030	175	7	2844	93	1	2155	31
Timothy (*Phleum pratensis* L.)	None	1	7119	87	1	4148	39	1	1849	14
		4	4576	115	7	1172	30	1	1598	14
	Abundant	1	6369	70	1	5429	79	1	7078	62
		4	4288	107	7	1981	65	1	5319	59
Red top (*Agrostis alba* L.)	None	1	7396	116	1	3016	26	1	1250	11
		4	5917	165	7	1667	45	1	1505	15
	Abundant	1	6773	78	1	5647	54	1	5016	57
		4	6587	201	7	2862	98	1	3789	61

RESULTS.—The data in table IV are presented primarily to emphasize the important differences in the amounts of nitrogen removed with various cutting treatments of the grasses in relation to their reserve content. GRABER, NELSON, LEUKEL and ALBERT (**8**) state that with alfalfa "nearly twice the amount of total nitrogen was removed in the top growth cut six times at succulent stages as in two cuttings taken at the seed stage." This, of course, occurred with alfalfa which prior to cutting was well stored with reserve foods. With grasses rather similar results have obtained particularly in 1926 when, prior to cutting, the reserves in all grasses were abundant and to a less extent in subsequent years when, with frequent cuttings, there were sharp declines in yields. The percentage of nitrogen in the grasses used in this experiment, roughly may vary from three to four per cent. in early succulent stages of growth to less than two per cent. at or near maturity. When the organic reserve of grasses are sufficient to maintain abundant productivity with frequent cuttings—as was true in 1926—the removals of such immature growth make for a very heavy draft on the available nitrogen supplies in the soil so that nitrogen itself may become the limiting factor in subsequent growth. However, when such deficiencies are avoided by abundant fertility or by heavy fertilization, the available carbohydrates are rapidly reduced and may become the limiting factors of growth under a drastic cutting or grazing treatment. This is well illustrated by the yields of 1928 where all the grasses were cut but once at or near maturity on July 7. With every grass which was fertilized the yields are decidedly less when they had been cut frequently in 1926 and 1927. Whether or not frequent cuttings may for a time result in a heavier draft on other elements of soil fertility was not ascertained in these trials, but has been established by many investigators.

ORR (**22**) finds "a seasonal variation in the mineral content of grasses. In Scotland they tend to be richest in July and become poorer with the advance of the season. This appears to be due not so much to the season *per se*, as to the stage of growth of the plant. At the period when it is growing most vigorously and when there is a maximum of leaf full of active protoplasm, the mineral content is at its highest. If by repeated cutting the plants are prevented from attaining maturity with the accompanying increase in fibrous material in the stems and reserve organic material in the seed, the period of high mineral content is prolonged." Similar results are indicated by HOPPER and NESBIT (**10**) from studies of the composition of prairie grasses in North Dakota.

While the nitrogen content of the grasses of this experiment (table IV) was not affected greatly by fertilization in 1926 probably due to an abundance of available nitrogen in the soil, the precentage of nitrogen in the

grasses in 1927 and 1928 were, with very few exceptions, much higher where nitrogenous fertilizers were applied. Such increases occurred at mature and immature stages of growth. Cutting treatments also had a subsequent effect on the nitrogen content of the grasses. In every case except one, the percentage of nitrogen was considerably higher on July 7, 1928, in the grasses cut frequently in 1926 and 1927 than in those which had been cut at or near maturity in the two previous years. This indicates a carbohydrate-nitrogen ratio, which was more favorable for vegetative growth than it was for the production of seed. There were external indications of this in the appearance of the plats of all grasses in 1929 and 1930, but this was particularly true of fescue, blue grass and timothy.

Ecological observations.—That "low" reserves in grasses resulting from frequent cutting and low nitrogen supplies in the soil tend to lower the competitive efficiency of grass and thus favor the entrance of foreign plants, especially those with a decumbent habit of growth was made evident by the general prevalence of scattered plants of white clover (*Trifolium repens*) in all the "low" reserve and unfertilized grasses (particularly fescue and red top) during 1928. Heavy nitrogen fertilization, however, practically eliminated the infestation of this clover. Timothy, low in reserve, was also abundantly infested during the summer of 1927 with thyme-leafed spurge (*Euphorbia maculata*), a decumbent plant which largely escaped mowing, but at times added considerably to the yields. The absence of all types of foreign growth was conspicuous in the plants of grasses with "high" reserves and abundant fertility.

Observations made on June 28, 1929, show again how effectively heavy nitrogenous fertilization prevented the invasion of white clover in both the "high" and "low" reserve grasses. With the absence of fertilization the plats of grasses "low" in reserves were abundantly infested with this clover, but it was much less prevalent in the unfertilized plats of high reserve grasses. Owing to the abundance of this legume in the unfertilized plats during 1929 the collection of data on yields was abandoned.

Growth recovery.—As was true with the blue grass in experiment I, all the grasses used in experiment II were slow to recover after cutting at or near maturity and particularly where such grass had been heavily fertilized. The grasses on both the unfertilized and fertilized plats which had been cut at frequent intervals in 1926 and 1927, regenerated new growth with greater uniformity and thickness after cutting on July 7, 1928, than grasses cut only at or near maturity. With the latter a tufted type of turf was particularly evident in the plats of fescue and red top, and to a much less extent in timothy and blue grass.

A tufted growth in grasses is due largely to short rhizomes and other forms of stem growth which radiate outward and which have a tendency for

annual increments in numbers and space occupied. When such grasses are allowed to mature or approach maturity before cutting it appears as if the number of individual plants which survive competition with each other is much less than when the aerial competition is reduced by cuttings. This is true if such cuttings are at a level and within limits of frequency and duration which do not greatly imperil the existence of these plants by exhaustion of reserve foods and the ingress of weeds. With fewer plants per unit area the stand is compensated for in thickness by the more rapid and abundant growth from such plants after recovery has occurred. As previously explained, however, recovery in growth after cutting at maturity may be delayed considerably due to the etiolation and death of many basal shoots and leaves occasioned by the density of growth previous to cutting. To the contrary, when top growth is retarded by ordinary deficiencies in reserves, fertility or moisture, injury to the active basal leaves and buds from the growth above is lessened so that recovery after cutting may proceed much more uniformly even though less abundantly as far as ultimate productivity is concerned. Such conditions give the impression that frequent cuttings tend to "thicken the turf" of grasses. If such thickness is measured by the number of individual shoots or leaves per unit area and by the rate and uniformity of recovery after cutting (important factors in the management of lawns and golf courses) the contention may hold, but if this statement includes the concept of productivity as measured by the total amount of top growth produced in an extended period, it may be very erroneous.

Top growth and root development of grasses and legumes grown in greenhouse under various systems of defoliation

The efforts which were made to determine the relative amounts of subterranean growth of the several grasses grown under field conditions in experiments I and II were seriously complicated by the great difficulty of separating live rhizomes and fibrous roots from those which were dead and also from partially decomposed leaves which constituted a considerable part of the turf. To obviate these obstacles, cultures of various grasses and legumes were grown in pots in the greenhouse. This made possible certain degrees of control with reference to the external environment which eliminated many field complications occasioned by drought, the ingress of weeds, insect injury, etc. When food reserves of plants are lowered by cutting treatments of the top growth, the losses in yield under field conditions which occur subsequently, may be due not only to the limitations of growth occasioned by the deficiencies of organic foods in the plants, but to many indirect influences such as a greater susceptibility to drought and winter

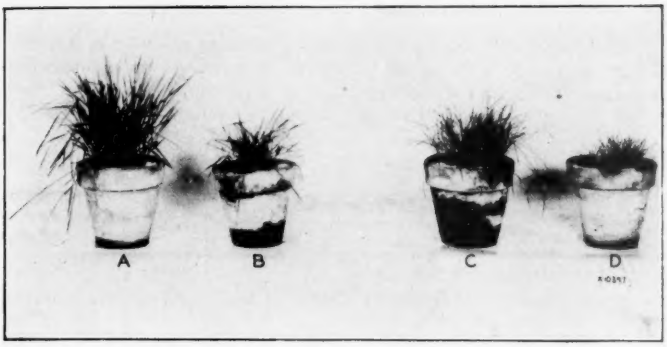

FIG. 4. Growth of red top and fescue grasses after frequent and infrequent cuttings. The grasses in pots B (red top) and D (fescue) were clipped six times between September 28 and November 13, 1928, while those in A (red top) and C (fescue) were clipped only once on November 13, 1928. The above photo was taken on December 1, 1928, after 17 days of growth in greenhouse.

injury, a lessened absorptive capacity for nutrients necessary for growth, a decrease in nitrogen supply in the soil and the invasions of weeds and other foreign growths. These complications can be avoided in greenhouse cultures so that the specific influence of the organic supply on subterranean and top growth can be more adequately measured. This was attempted in experiments III, IV and V. See also fig. 4.

EXPERIMENT III

On June 26, 1928, eight five-inch pots were filled with fertile silt loam soil. Four of these were sown with 0.6 gram each of blue grass seed, and four with 0.4 gram each of red top seed. On the same day 16 seeds of timothy were sown equidistantly in each of 9 eight-inch pots filled with similar soil. The pots were watered uniformly, and after 24 days of growth in a greenhouse, a clipping treatment was begun on 5 pots of timothy, 2 of red top and 2 of blue grass. The clippings were made one inch above the soil surface and in case of blue grass consisted entirely of leaf growth. The clippings of timothy and red top were for the most part of leaves but included some stem tissue. Seven such clippings were made on the following dates: July 20 and 25, August 2, 6, 11, 18, and 21, 1928. The grasses in the remaining pots were allowed to grow until August 24, 1928. On this date all plants were removed from pots with running water. The roots were separated from the tops. The dry weights of roots from clipped and unclipped plants at the close of a period of growth of 59 days from date of seeding are given in table V.

TABLE V

EFFECT OF CLIPPING LEAF BLADES OF SEEDLING PLANTS OF BLUE GRASS, RED TOP AND TIMOTHY ON THE AMOUNT OF ROOT GROWTH. PLANTS GROWN IN GREENHOUSE FROM SEED SOWN IN POTS OF FERTILE SILT LOAM SOIL ON JUNE 26, 1928. DRY WEIGHTS OF ROOT GROWTH DETERMINED ON AUGUST 24, 1928. TOTAL PERIOD OF GROWTH 59 DAYS

KIND OF GRASS	TREATMENT OF LEAF GROWTH	NO. OF POTS	NO. OF PLANTS	DRY WEIGHT IN GRAMS OF ROOTS OF 100 PLANTS
Blue grass (*Poa pratensis*)	Not clipped	2	293	0.64
	Clipped 7 times	2	336	0.08
Red top (*Agrostis alba*)	Not clipped	2	434	1.82
	Clipped 7 times	2	393	0.10
Timothy (*Phleum pratense*)	Not clipped	4	44	7.89
	Clipped 7 times	5	69	0.55

Since nearly all the organic substances of the subterranean parts are first synthesized in the tops, a larger root growth from the unclipped plants was to be expected, but the increase from 737 to 1720 per cent. is much more than was anticipated. It is significant to add that the 293 blue grass plants not clipped before they were removed from the pots produced in addition to their root growth, 75 underground lateral rhizomes weighing 0.13 gram. The plants of clipped blue grass produced no rhizomes.

EXPERIMENT IV

Thirty pots (five inch) were filled with fertile silt loam soil which had been sifted to remove all coarse particles in order to facilitate later removal of fibrous root growth of the sweet clover, alfalfa, alsike clover, red clover, white clover, red top, blue grass and fescue which were sown broadcast and separately in such pots on August 28, 1928. The seedling plants of grasses and legumes were allowed to grow in the greenhouse for a month when clippings of the leaves (not stems) from the plants growing in one-half of the pots were made on September 28, October 4 and 13, 1928. On October 24, 1928, all the plants, clipped and unclipped, were carefully removed from the pots with running water, and the roots separated from the tops. Dry weights of the total top and root growth during a period of 57 days is given in table VI. The plants were watered uniformly by sub-irrigation, the pots being kept in water tight wooden flats. Nitrogen in the form of ammonium sulphate was added to the water applied so as to maintain a vigorous growth.

The pronounced decrease evident in root growth of all plants with three clippings of the foliar parts makes a detailed discussion of the data in table

TABLE VI

EFFECT OF CLIPPING LEAF GROWTH OF SEEDLING PLANTS OF RED CLOVER, SWEET CLOVER, ALFALFA, ALSIKE CLOVER, WHITE CLOVER, RED TOP, BLUE GRASS AND FESCUE ON THE AMOUNT OF TOP AND ROOT GROWTH DURING A PERIOD OF 57 DAYS OF GROWTH IN GREENHOUSE FROM DATE OF SEEDING ON AUGUST 28, 1928, TO DATE OF REMOVAL OF PLANTS FROM POTS ON OCTOBER 24, 1928

PLANT	NUMBER OF POTS	NUMBER OF PLANTS	NUMBER OF CLIPPINGS PRIOR TO OCT. 24	GRAMS OF TOTAL DRY ROOT GROWTH PER 100 PLANTS ON OCT. 24, 1928	GRAMS OF TOTAL DRY TOP GROWTH PER 100 PLANTS, INCLUDING THAT PRESENT ON OCT. 24, 1928
Red top	2	46	0	8.65	4.83
	2	62	3	2.17	0.33
Fescue	2	68	0	6.15	1.18
	2	59	3	1.58	0.25
Blue grass	2	66	0	3.61	0.72
	2	29	3	1.44	0.24
Sweet clover	1	8	0	35.13	3.92
	1	11	3	5.63	0.64
Alfalfa	2	24	0	13.46	3.00
	2	21	3	6.57	0.68
Alsike clover	2	39	0	10.20	1.65
	2	15	3	3.29	0.39
White clover	2	53	0	10.09	1.77
	2	31	3	2.37	0.28
Red clover	2	46	0	11.00	2.31
	2	36	3	3.38	0.27

VI unnecessary. With the loss of photosynthetic area and the utilization of reserves in the regeneration of new growth following the three removals of leaves, the amount of top growth produced with 57 days of growth following seeding was also very much less for the clipped plants.

EXPERIMENT V

Plants of blue grass, fescue and timothy were established in eight-inch earthen pots (six-inch pots in case of fescue) filled with a mixture of quartz and sifted fertile loam soil. The seed was sown on November 1, 1928. The number of plants which became established in each pot under the conditions of greenhouse culture varied from two to nine although in 76 per cent. of the pots the number of plants ranged from four to six inclusive. The pots were kept in water tight wooden flats which made possible uniform

applications of water to each plant as well as uniform amounts of potassium nitrate and ammonium phosphate which were added from time to time in solution so as to provide for optimum growth.

All the plants grew for 81 days, when on January 20, 1929, the top growth was cut one inch above the soil surface. On this date the plants were well established, but with no external evidence of inflorescences, except in the case of timothy where several spikes appeared. The older leaves of fescue had made a healthy dark green growth of from 4 to 6 inches in length, those of blue grass 6 to 9 inches while the older timothy stems had grown from 8 to 12 inches. The top growth of the plants in four pots of fescue, three of blue grass and four of timothy were cut again in a similar manner on January 30, February 5, 14, and 23. On the last date a second cutting of top growth was taken from the plants in the remaining three pots of fescue, three of blue grass and four of timothy. The five cuttings on part of the plants and the two cuttings on the remainder will be referred to hereafter as pre-cuts. Such cuttings were dried and weighed and the data are recorded in table VII.

On March 7 and March 12, 1929, clippings were again made on the grasses in all pots after which they were allowed to grow for a period of 43 days before the last cutting was taken on April 24. These cuttings were dried and weighed and will be referred to as recovery-cuts. On April 24, neither fescue nor blue grass had produced flower stalks while a few flowering spikes of timothy were present. The amount of top growth separated from the roots on April 24 is indicated in table VII. It consisted almost entirely of leaves in the case of fescue and blue grass, but of stems and leaves in the case of timothy.

On the day of the last cutting (April 24) the plants were removed from the pots and washed. The roots were separated from the top growth, and from the rhizomes in the case of blue grass, while with fescue the very short rhizomes were regarded as top growth and the fibrous root growth only was separated. Timothy roots were separated at the base of the stems.

The total period of growth for all plants from November 1, 1928, the date of seeding, to April 24, 1929, the date of the last cutting of top growth, and the separation of roots from stems was 175 days. During the first 81 days, the plants were unmolested; during the second 34 days, the pre-cuts were made, and during the last 60 days, the three recovery-cuts were taken.

Even though the grasses were well established with a root development of 81 days of uninterrupted growth before cutting treatments were begun, five pre-cuts of top growth yielded only one-third to one-half the amount of dry matter obtained from two pre-cuts. The five pre-cuts were taken (table VII) on January 20 and 30, February 5, 14 and 23, 1929. The two pre-cuts were taken at the beginning and the close of this period of 34 days.

TABLE VII

QUANTITATIVE EFFECTS OF FREQUENT AND INFREQUENT CUTTINGS OF FESCUE, BLUE GRASS AND TIMOTHY APPLIED AFTER 81 DAYS OF UNINTERRUPTED GROWTH IN GREENHOUSE. GRASSES SOWN IN POTS NOVEMBER 1, 1928

PLANT	NO. OF POTS	NO. OF PLANTS	DRY WEIGHT OF PRE-CUTS IN GRAMS 1929						DRY WEIGHT OF RECOVERY-CUTS IN GRAMS 1929			DRY WEIGHT OF ROOTS IN GRAMS 4/24, 1929
			1/20	1/30	2/5	2/14	2/23	Total	3/7	3/12	4/24	
Fescue	4	21	1.477	0.335	0.152	0.121	0.106	2.191	0.180	0.083	9.95	3.56
	3	17	1.620				4.780	6.400	2.210	0.413	22.98	8.87
Blue grass	3	19	2.303	0.465	0.300	0.333	0.295	3.696	0.736	0.325	21.01	13.81*
	3	17	2.140				6.570	8.710	3.025	0.623	30.74	18.95**
Timothy	4	16	2.633	0.550	0.252	0.215	0.435	4.085	0.511	0.192	19.74	5.39
	4	16	2.382				6.417	8.799	1.554	0.592	33.02	8.25

* In addition to this root growth the 19 plants of blue grass produced 276 rhizomes weighing 3.64 grams (dry basis) and the 17 plants ** of blue grass developed 365 rhizomes weighing 6.45 grams (dry basis).

On March 7, twelve days after the last pre-cut was made, the first recovery-cut was taken on all the plants. Five days later (March 12) a second recovery-cut was taken. Both of these recovery-cuts consisted of succulent leaf growth varying in length from 1 to 2 inches. The grasses with only two pre-cuts made a growth recovery which quantitatively was several times that of the recovery-cuts on grasses with five pre-cuts. A third recovery-cut was made forty-three days later on April 24, 1929. At this time, the amount of top and root growth was much smaller with grass plants having been given five pre-cuts instead of two. The three additional pre-cuts made on January 30, February 5 and 14 greatly decreased subsequent productivity and constituted the only controlled factor of variance in the experiment. The greater number and quantity of rhizomes in case of blue grass given two pre-cuts is substantial evidence of the ready influence which cutting treatments of top growth may exert on subterranean development.

Summary

Marked quantitative responses of root, rhizome and top growth of various grasses (blue grass, red top, fescue and timothy) grown under field and greenhouse conditions are correlated with cutting treatments which affect the internal environment. Not only did the amount of subterranean growth and total weight of top growth, ultimately, tend to vary inversely with the frequency of defoliation but reduced growth sometimes occurred for several months subsequent to excessive defoliations. It is clearly evident that the productive capacity of grasses is not only dependent upon adequate supplies of available nutrients and moisture combined with favorable light and temperature conditions but also upon the food reserves of the plant.

When photosynthesis is interrupted by frequent removals of top growth the limitations of subterranean development may involve greater susceptibility to drought, lessened absorptive capacity and increased winter and insect injury.

The capacity of the plant for maintaining a surplus of organic foods is generally enhanced when its morphology or its inedibility or unpalatability or the type of grazing or cutting is such that a considerable portion of the photosynthetic area escapes removal. Such plant characteristics along with variations in the duration of periods of succulent growth explain, in part, the wide differences in responses of various agronomic plants to specific removals of top growth.

Frequent and close removals of the succulent top growth of grasses having abundant reserves make for a heavy draft on the supplies of available nitrogen in the soil so that the first important factor of growth limitation

may be nitrogen deficiency. When regeneration is constantly stimulated by a fertile soil or by abundant mineral and especially nitrogenous fertilization, the carbohydrate reserves are rapidly consumed and with slight opportunity for replenishment they often become the principal factors limiting growth.

When an abundant growth of blue grass occurs with fertilization and delayed cutting, the recovery after such cutting is often slow and uneven due, in part, to etiolation and the death of many basal leaves occasioned by the dense growth above. Such delay in recovery may, under favorable climatic conditions, encourage the ingress of certain weeds and may prove significant in the management of heavily fertilized pastures or of turfs and lawns where uniformity is desired.

Organic food reserves have a significant ecological relationship, especially with reference to the flora of grasslands. Aside from the occurrence of weeds with delayed cutting, the maximum competitive efficiency of beneficial grasses, as measured by invasions of other plants among them, occurred generally, when optimum fertility was combined with those practices of cutting or grazing which maintained a productive level of reserve foods in such grasses.

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SOME REACTIONS OF THE BANANA TO PRESSURE, GRAVITY, AND DARKNESS[1]

ALEXANDER F. SKUTCH

(WITH FOURTEEN FIGURES)

I. Introduction

The reactions of growth of the typical dicotyledonous plant to the directive stimuli of its environment, especially to light and gravity, are described in every elementary text-book of botany. The examples chosen to illustrate these responses are almost always plants with a terminal growing point and elongated internodes. When we try to picture the behavior, under the same conditions, of a monocotyledonous plant provided with a massive false-stem, its true stem condensed to the utmost possible degree, and its growing point buried beneath a dozen layers of thick leaf bases, we are faced with a lack of precise information. Assuming, for the moment, the same inherent physiological tendencies in the rapidly growing portions of the banana or a similar plant, that we recognize in the shoot of a mint or a composite, it is difficult to imagine how these tendencies can find expression, since the young organs are imprisoned in a massive panoply of protecting leaf-bases. What, for example, would be the behavior of a banana plant placed in a horizontal position? The following experiments were undertaken in an attempt to clear up this problem.

In an earlier paper (**6**) I described the structure of the false-stem of the banana, but it may be convenient to characterize it briefly here. The stem of a plant which has not yet begun to "shoot," or produce its inflorescence, is a massive rhizome, expressively termed a "bulb" by the banana grower, the diameter of which usually about equals or sometimes exceeds its height (fig. 7). On the lateral and upper surfaces of this organ the leaf-sheaths are inserted in close contact with each other. In the center of the leaf-bases is the upwardly directed terminal growing point of the rhizome, which is very low, or even sunken beneath the portions of the rhizome on either side of it. The closely-overlapping leaf-sheaths form a tall and slender false-stem, the structure of which is best explained by reference to figure 1. The young leaves formed at the growing point must perform a long journey upward through the center of this false-stem before they can finally expand. Only when the plant reaches maturity and prepares to flower does the true stem begin to elongate and push up in the midst of the leaf-sheaths. It then forms a columnar shoot, rather thick, but totally unable to support itself when deprived of the enclosing leaf-sheaths. Upon it are borne, at intervals

[1] Botanical Contribution from the Johns Hopkins University no. 110.

FIG. 1. Diagrammatic cross-section of false-stem. For clearness, sheaths have been separated from each other in drawing, actually they are in closest contact. Prominent lacunae occupy the greater portion of their interior. In center is lamina of emerging leaf. A large false-stem is made up of many more leaf-sheaths than are shown here.

increasingly distant, the largest leaves produced by the plant. Finally it emerges from the top of the false-stem and gives rise to the familiar pendent inflorescence. It is only with the younger plant, whose stem has not yet begun to elongate, that the present experiments deal.

II. Distribution and rate of growth

In order to understand the reactions of the banana to darkness and gravity, it is essential to know the rate and especially the distribution of elongation of the leaf sheaths. The sheath of the newly appearing lamina is in all cases elongating the most rapidly, but the sheaths of several of the older leaves surrounding this are at the same time making considerable growth. The prolonged though slight growth at the bases of the older sheaths will prove of great importance when we come to consider the geotropic erection of the false-stem.

The differentiation of the leaf of the banana is basipetal (**7**). When the lamina first peeps forth from the top of the false-stem (see **6**, fig. 9) it is full grown. The sheath, which up to this time has lagged behind in growth, now begins to elongate rapidly. The greatest growth occurs in its basal portion, which has remained the least differentiated. The localization of growth in the base of the leaf-sheath here described rests solely upon anatomical evidence, since it has been found impossible to uncover portions of a rapidly elongating sheath, by removing segments of the older, enclosing sheaths, and mark it for growth measurements, without seriously disturbing its growth

(see section III). However, the vertical separation of the transverse septa or diaphragms (which differentiate very early) in various regions of the sheath, when compared with their separation in corresponding regions of a mature sheath, affords a ready method of determining the regions of greatest elongation. This method has proved of importance in some of the experiments which follow.

In determining the rate of elongation of the various leaves, the following method was employed. At the top of the sheath of the fifth leaf from the apex of the false-stem, a scratch was made extending from its margin on to the outer face of the next sheath within. The point of a scalpel, dipped in India ink, was found most convenient in making an enduring and easily recognized mark. The successive leaves were similarly marked, and the tip of the emerging leaf was measured in relation to a fixed point on the youngest fully expanded leaf. Thus the elongation of any leaf during a given period may be found by adding the partial increments of all the sheaths below.

The absolute rate of emergence of the leaf depends, other things being equal, upon the height of the plant, for the taller plants have longer leaf-sheaths, and these in turn possess longer growing zones. The growth of the emerging leaf is extremely rapid. MAXWELL (**3**) found a maximum rate of 21 cm. per day, while the writer, probably because he measured larger plants, recorded a maximum rate of elongation of the sheath of 35 cm. in 24 hours, on a vigorous plant of *Musa sapientum* subspecies *seminifera* with a false-stem about 3 m. high, growing near Almirante, Panama, during the rainy season.

Taken from the time the leaf peeps forth from the top of the false-stem, the sheath shows an increasing daily rate of elongation until a day or two before the lamina begins to unfurl (see fig. 2). Before unrolling, the lamina stands up above the false-stem like a slender rod. The process of unrolling begins at the apex, and for plants growing rapidly in a favorable environment requires from 1 to 4 days for completion, according to the size of the leaf and the vigor of the plant. During the period of unfurling, the daily rate of elongation of the sheath falls rapidly, then sinks more slowly until, when the following leaf appears, it is making 4–6 cm. growth per day (in plants 2–3 m. high under good growing conditions). The sheath continues to elongate more slowly for several weeks, and even after the second subsequent leaf has fully expanded, it usually makes 1 or 2 mm. growth per day, while a very slight growth can be detected after the third subsequent leaf has expanded. To state the same facts somewhat differently, at any given time the sheath of the emerging, still furled leaf shows the maximum rate of elongation, the first leaf below elongates 1–6 cm. daily, according to the period which has elapsed since its own expansion, the second leaf below

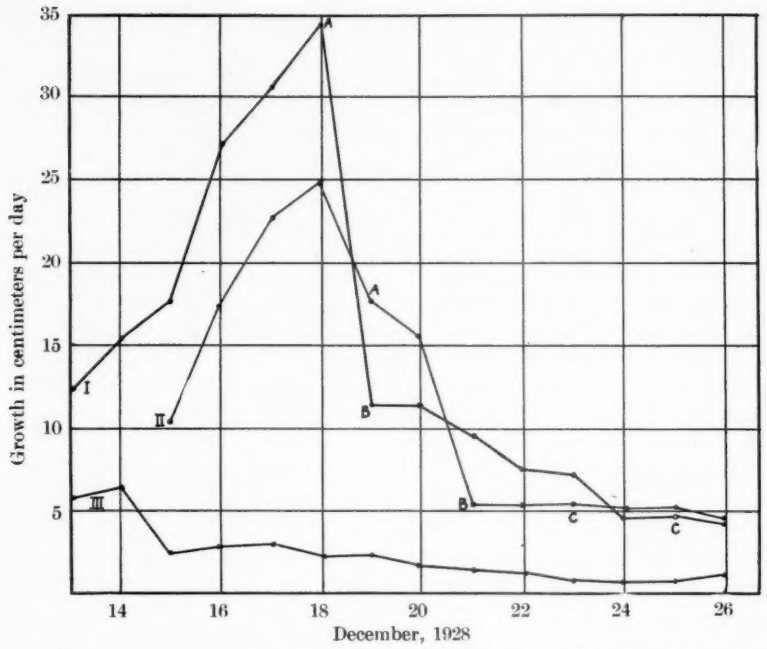

FIG. 2. Rate of growth of banana leaves. Graphs I and II give growth of two leaves from time of first appearance at top of false-stem (I on Dec. 12, II on Dec. 14) to after appearance of following leaf. A = beginning, B = completion of unfurling of lamina, C = time of appearance of next leaf. Graph III gives growth of leaf preceding I on same plant during same period. Graph I as far as C, then graph III accordingly give a fair picture of growth of leaf for a 4-week period.

elongates a few millimeters, while the third and sometimes the fourth show a slight and irregular growth. During the period from 12:30 P. M. December 17 to 12:30 P. M. December 18, 1928, the leaves of four plants grew as follows:

Plant	*A*	*B*	*C*	*D*
Emerging leaf (still furled)	170 mm.	346 mm.	349 mm.	163 mm.
Next older leaf (completely expanded)	27	22	19	13
Second older leaf	3	1	8	1
Third older leaf	1	0	3	0

III. Pressure of growth

Great pressure is required to force the emerging leaf upward through the false-stem of a large plant. Each sheath fits tightly over those within it, and there is no wasted space. A leaf emerging from the top of a false-

stem 3 m. high is closely embraced by the sheath of the next-older leaf throughout this distance. If it is elongating at a rate of 30 cm. per day, while the sheath of the enclosing leaf is pushing upward at the rate of 5 cm. in 24 hours, then the former must slide through the latter for a distance of 25 cm. Since the growth of both sheaths is largely basal, this relative rate of motion holds good throughout most of the 3 m. stretch. The sheath of the second leaf, growing 5 cm., must slide upward through that of the third, and the third through the fourth, as long as growth continues. All surfaces of contact between the sheaths are covered with a smooth cuticula, which eases the sliding motion.

The work which the leaf must perform in emerging from the false-stem is still further increased by the necessity to spread apart all of the older sheaths as it pushes upward, because it expands gradually toward the base. Its action is that of a gently tapering entering wedge or cone. The expansion of the false-stem by the emerging leaf necessitates the lateral movement of both faces of each older sheath over all adjoining faces. In addition, these sheaths must experience a considerable change in the shape of their cross-section as they are pushed outward (compare the outer with the inner sheaths of fig. 1). The sheaths remain to a certain extent plastic and capable of slow growth-adjustments for a long time after they have ceased growth in length. The gradual maturation of the lignified mechanical elements of their tissues, which are not completely developed until the sheath comes to lie at the surface of the false-stem, as a result of the dying and shedding of those exterior to it, is significant in this connection (**6**, pp. 351–352).

If one cuts out a length of false-stem of say 30 cm. or more and tries to push or pull forward the portion of the emerging leaf included in it, he will find all of his strength of little avail in budging it. Its movement is possible only as a result of the slow, uniform adjustment of all the component parts of the false-stem to the growth pressures prevailing in it. The whole dynamic system is most delicately adjusted and is easily upset. The sheaths of the emerging leaves of four large plants were exposed by the removal of a rectangular section from all of the sheaths exterior to them, at a point somewhat below mid-height of the false stem (fig. 3). The operation was performed carefully, and the sheath of the emerging leaf was not injured. The cavities were filled with moist absorbent cotton, and wrapped to prevent the drying of the exposed surfaces. The experiments were performed during a rainy period, and the plants showed no indications of wilting as a result of the loss of a portion of their conducting system. The length of sheath thus exposed was 13–20 cm., the plants having false-stems 2.1–2.6 m. high. In no case did the leaf with a portion of the sheath exposed complete its emergence, although that with only 13

FIGS. 3–4. FIG. 3 (left) effect of exposing sheath of emerging leaf (see text, p. 77). Operation performed April 3, photographed April 5. FIG. 4 (right) same plant on June 4, showing production of forked false-stem.

cm. exposed pushed upward 60 cm. after the operation. In all of the others, the exposed length of the sheath began to bulge into the cavity formed in the side of the false-stem (fig. 3). The sheath, devoid at this stage of lignified elements, but consisting almost entirely of delicate thin-walled cells and large lacunae, was not sufficiently rigid to transmit the thrust of the growing base to the more apical regions of the leaf. The subsequent leaves burst through the top of the loop thus formed, and eventually a plant with a forked false-stem was produced (fig. 4), a phenomenon which I have never seen under natural conditions. Occasionally, however, one finds an inflorescence which has burst through the side of the false-stem, instead of emerging from the top, because some abnormal condition in the upper portion of the false-stem prevented its appearance in the usual manner.

The pressure, under which the tissues in the basal portion of the sheath of an emerging leaf are laboring, may be demonstrated in another way. Three large plants, with false-stems between 3 and 4 m. high, were marked for growth measurements as described above, and the growth determined at 30 minute intervals for 3 hours. The false-stem was then severed cleanly in the middle by a long, sharp knife. Almost instantaneously the cut end of the most rapidly growing sheath sprang up a few millimeters above the surface of the stump, a result of the release of the pressure against which it was growing. The total elongation of the emerging leaf was now determined at 15-minute intervals by adding (1) the emergence above the cut surface of the stump, (2) the emergence from the cut end of the top of the plant, (3) the emergence from the top of the false-stem in the normal manner. The portion included in the severed apical half of the plant was now pushing out at both ends, despite the fact that it was dissevered from its water supply, but the basal half of course made the greatest growth.

	Rate of growth during 3 hours before operation	Rate during first 15 minutes after operation	Increase in rate
Plant 1	14.7 mm. per hour	72 mm. per hour	4.9 times
Plant 2	12.7 " " "	84 " " "	6.6 "
Plant 3	12.0 " " "	108 " " "	9.0 "

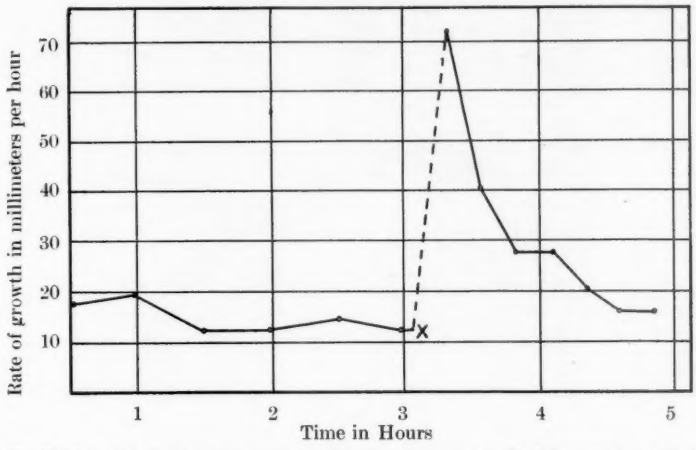

FIG. 5. Effect of relieving pressure on sheath of emerging leaf by cutting off top of false-stem on its rate of growth. X marks the point when the false-stem was severed.

After the first fifteen minutes the growth rate gradually fell, but only after 2–3 hours did it decrease to its value previous to the operation (see fig. 5).

IV. Reactions to gravity

Erection of seedlings

Vigorous young seedlings readily execute geotropic curvatures. On March 9, at 8 A. M., a gardener's flat containing several dozen seedlings of the abacá (*Musa textilis* Née) was set on edge, so that the plants were horizontal. The majority of the plants were 5–7 cm. in total height, and had produced 6–7 plumular leaves. By the afternoon of March 11, they had

Fig. 6. Erection of seedling abacá (*Musa textilis*) in 56 hours after being placed horizontally.

bent upward until their false-stems formed angles of 80°–90° with the horizontal. The curvature had taken place at the base of the false-stem, and was produced by the growth of the leaf-sheaths (see fig. 6).

Erection of older plants

Banana plants of seedling origin when overturned preserve their ability to erect themselves from the base for a surprisingly long period, and the

largest plants tested had not yet lost this capacity. In the spring of 1929, a number of hybrid plants grown from seed, with false-stems ranging up to 2 m. in height, were available for experimental purposes. They were rooted in the ground, and were too large to be successfully transplanted to pots or boxes. Accordingly the soil on one side was gently loosened with a pitchfork, and the plants were slowly tilted over until they made an angle of 15°–45° with the ground, when the earth was again packed around the roots. The plants suffered little injury from this treatment, and remained fresh and turgid.

In the first experiment 19 plants, with false-stems ranging from 50 to 150 cm. in height, bent upward through 20°–50° in one week. In the second experiment, on April 8, 22 plants ranging up to 202 cm. in height were tilted over in the same manner, until they made angles of 20°–45° with the ground. During the first week most of them curved upward through 20°–45°, so that the most advanced now inclined only 5°–15° from the vertical. A number were now dug up for anatomical study. The basal portion of the plant was cut in half longitudinally along the plane of curvature, and the actual curvature presented by the section was measured as accurately as possible by the use of two rulers and a protractor (see table I). The plants which were allowed to remain in the ground were in a few weeks practically erect again, and continued to grow normally.

The largest (but not the tallest) plant used in these experiments had a false-stem 189 cm. high, and 20 cm. in diameter at the base (see fig. 8 and table I, plant 10). The total height to the tip of the leaves was 385 cm.,

TABLE I

EFFECTS OF SETTING BANANA PLANTS IN AN INCLINED POSITION
INCLINED ON APRIL 8TH, DUG UP AND MEASURED APRIL 16–18TH

No.	HEIGHT OF FALSE-STEM	TOTAL CURVATURE	CURVATURE OF FALSE-STEM	CURVATURE ASCRIBED TO RHIZOME	DIAMETER OF RHIZOME	THICKNESS OF CORTEX	
						LOWER SIDE	UPPER SIDE
	cm.				*cm.*	*cm.*	*cm.*
1	156	35°	22°	13°	13.0	3.1	2.1
2	93	45	30	15	7.5	1.8	1.2
3	145	35	27	8	9.0	2.5	1.4
4	202	34	17	17	15.3	4.0	2.7
5	190	37	23	14	11.5	2.6	2.1
6	131	39	27	12	7.1	1.8	1.0
7	126	40	28	12	7.4	2.1	1.2
8	118	30	14	16	6.6	1.8	1.4
9	89	25	15	10	5.5	1.4	1.2
10	189	30			20.0	4.4	3.7

and the largest leaf possessed a blade 173 cm. long. In 8 days, this plant changed its angle of inclination through 30°, from 45° to 75°. The weight of the aerial portions (false-stem and laminae) was 15 kilograms, and the center of gravity of the leafy stem was 92 cm. above the bulb. Unfortu-

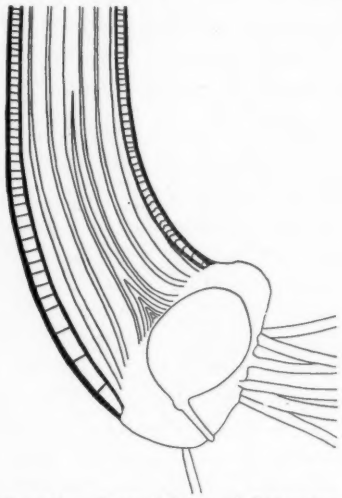

FIG. 7. Longitudinal section through base of false-stem and rhizome of banana hybrid (seedling origin) inclined at angle of 15° with horizontal March 6, dug up April 6, after it had curved upward about 70°. Note greater thickness of false-stem and cortex of rhizome on lower side. (× ⅓.)

nately the system is too complex to allow the computation of the pressure on different portions of the bulk from these data, but some idea of the magnitude of the work performed in erecting this stem may be gained from the observation that I was not strong enough to support the false stem at its original angle of inclination, when holding it at its base.

The mechanism of this geotropic curvature is complex; two distinct factors are involved in it: (1) the curvature of the bases of the leaf-sheaths and (2) the enlargement of the lower side of the rhizome, especially its cortex. While the participation of these two distinct regions in producing the total curvature is perfectly clear, the part played by each separately is in practice extremely difficult to determine. By measuring the bases of plants sectioned longitudinally, such as that represented in figure 7, I endeavored to determine the degree of curvature to be attributed to each region. The difficulties of measuring these two values separately are very great, and the values recorded in columns 4 and 5 of table I are presented

FIG. 8. Banana plant inclined at angle of 45° on April 8, photographed April 16, after it had bent upward 30°. Note curvature at base of false-stem.

with diffidence. The results indicate that from ¼ to ½ of the total upward movement is a result of the activity of the "bulb."

Confining our attention first to the rhizome, it is found that certain very definite anatomical changes are induced there as a result of placing the plants in the inclined position. The cortex becomes very much thicker on the lower than on the upper side (see table I). This is brought about by the enlargement and division of the large, thin-walled, starch-laden mature cells of the ground parenchyma on this side. In the outer cortex, they elongate most strongly in the direction parallel to the surface (and to the long axis of the plant) and divide by one or more transverse walls. In the inner region of the cortex, the direction of elongation is not so regular, the long axes of the cells becoming often more or less strongly oblique, and the positions of the cross-walls are correspondingly irregular. Even a

month after the plants were turned over, the partition walls formed subsequent to the operation were quite distinct, since the original cell wall was still clearly recognizable, and the internal division walls were thinner and more delicate. Compared with the cortex on the lower side, that on the upper side was composed of smaller cells with walls very noticeably thicker, which were not strongly elongated in any particular direction and, except in rare cases, showed no evidence of the recent division of the mature cell. The exhaustion of starch on the lower side of the rhizome was very striking.

TABLE II

INCREASE IN THICKNESS OF LOWER SIDE OF FALSE-STEM PLACED IN INCLINED POSITION

PLANT NO.	THICKNESS, LOWER SIDE	THICKNESS, UPPER SIDE
	cm.	*cm.*
11	4.2	3.2
12	8.4	7.1
13	2.4	2.5
14	3.4	3.0
15	3.9	3.3
16	4.1	3.4
17	3.5	2.7
18	3.3	2.9
19	3.1	2.6

The starch grains here were few in number and much smaller than those in corresponding regions of the upper side. WARNER (9) found an increase in reducing sugars on the lower side of horizontally placed shoots of *Helianthus annuus* and *Silphium perfoliatum*. No tests for sugars were made in the banana rhizomes, but it is probable that the starch was converted into sugar, a portion of which at least was utilized in performing the work of raising false stems.

In the region of curvature, the false-stem was distinctly thicker on the lower (convex) than on the upper (concave) side (see table II and fig. 7). The increase in thickness of the leaf-sheaths lying on the lower side is a result of the enlargement, without division, of their component cells. Since the ground parenchyma was already very thin-walled, no decrease in the thickness of the walls of these cells could be observed. The disappearance of starch grains from the cells in the leaf-sheaths on the lower side of the false-stem in the region of curvature was very pronounced. In sheaths laterally situated, it was found that the lower flank was far poorer in starch than the upper flank of the same sheath. It is of interest that the grains in the "starch-sheaths" surrounding the vascular bundles showed no ten-

dency to diminish, even when the starch was practically exhausted from the other cells in the same region of the sheath. Evidently these persistent grains serve as statoliths.

The geotropic erection of the false-stem is not brought about merely by the agency of the sheaths of the two or three inner, actively growing leaves. That these leaf-sheaths are capable of exerting great pressure, at least under certain circumstances, will be made evident in the next section, but it is difficult to understand how, with their soft tissues and the internal position which places them at a mechanical disadvantage, they would be able to exert the pressure necessary to erect the large plants in question. The one or two outermost sheaths on the lower side, the laminae of which are dying or dead, are sometimes split neatly across the thick basal portion, giving a vivid sense of the great forces displayed in the upward curvature of the false-stem. At other times, they may slip over the inner sheaths without becoming torn. But aside from these, all of the sheaths on the lower side (their number varies with the age of the plant) take an active part in the curvature. This is indicated by several circumstances. (1) The great separation of the transverse septa in the base of these sheaths shows that they have elongated in this region. (Compare the interval between these septa in the sheaths on the upper and lower sides of the plant in figure 7). (2) When the false-stem is cut across in this region, the outer sheaths on the lower side elongate, indicating that they were in a state of compression, and not passively stretched. (3) These sheaths are thicker than corresponding sheaths on the upper side, and are decidedly poorer in starch grains, evidence that active changes have taken place in them.

In the region of curvature, the fibres, though thick-walled in the outer sheaths, are generally not lignified. The hypodermal cells likewise do not become lignified at this level. Thus the stretching of the older sheaths is more comprehensible.

BÜCHER (**1**) described under the term geotrophism the anatomical changes which occur in an orthotropous, herbaceous shoot, still capable of growth, when placed in a horizontal position and its erection forcibly prevented. Under these conditions, the cells of the collenchyma, bast and wood on the lower side acquire greater diameters than in corresponding portions of a normal shoot, while their walls remain notably thinner, at the same time these cells on the upper side show a reduced diameter accompanied by the thickening of their walls to a degree considerably above the normal. The whole cortex on the lower side becomes abnormally enlarged. These changes, which have been observed in a large number of dicotyledonous hypocotyls and epicotyls, appear to be quite general, but apparently are not appreciable in shoots which are free to bend upward under the influence of gravity. SCHWARTZ (**5**) has recently given a comprehen-

sive review of the literature on this subject. The increase in the size of the parenchyma cells of the sheaths and cortex on the lower side of inclined banana plants is clearly a related phenomenon. The great weight of the plants themselves causes the increase in cell size without the prevention of curvature by any external restraint.

Erection of transplanted suckers

When the buds situated on the side of the old rhizome, below the insertion of the living sheaths, begin to elongate, they at first grow through the soil in a horizontal direction. At this period they produce leaves consisting of bladeless sheaths, which gradually merge into leaves with reduced, linear laminae and thick, prominent sheaths. When the young sucker reaches a certain length, which varies considerably in different varieties, it begins to bend upward. The erection of the sucker is initiated by the leaf-sheaths, which curve upward with considerable force, raising and cracking the soil above them. The rhizome of the sucker more gradually follows the young false-stem in assuming an upward direction of growth, and enlarges into the "bulb" of the new plant.

One of the methods sometimes employed in the propagation of bananas is to sever the sword suckers from the parent rhizome and, after trimming away the laminae of the leaves, to set the bulb into the ground. If such propagating material is allowed to lie in the field for some time before planting, in wet weather it usually continues to grow slowly. Occasionally one finds, in a sucker which has been lying for several weeks in a horizontal position, that the heart leaves, in their efforts to grow in a vertical direction, have burst through the side of the old false-stem and begun a new false-stem at right angles to it.

In order to study this behavior more thoroughly, on February 14, 1929, fifteen young suckers, with false-stems between 30–100 cm. high, were dug with care, so as not to injure the false-stem. These suckers had already formed massive bulbs of their own (fig. 10). All of the visible lateral buds were cut away, in order to restrict the new top-growth of the suckers to their terminal growing point. The suckers were set in the ground inclined at angles of 10°–50° with the horizontal. Since all of the roots and none of the leaves were cut away, growth was effectively prevented until a new root system could be established. The object of the procedure was to retard the geotropic erection of the false-stem, so that when the young leaf rudiments resumed active growth, they would find themselves imprisoned within an inclined false-stem composed of sheaths which had lost the capacity of upward curvature. During the month which followed the transplanting, most of these plants succeeded in effecting more or less upward curvature, but their growth was retarded by lack of water, and they still remained

considerably inclined. No new growth of the tops was apparent, but meanwhile a new root system had become established. On March 11, the young leaves of one of the suckers burst through the upper side of the old false-stem, just above the bulb. In freeing themselves, they ruptured five living sheaths. The two oldest sheaths of the new false-stem, which had evidently exerted the greatest pressure, had torn off their laminae in escaping, leaving them imprisoned in the upper portion of the old false-stem. The pressure which it was possible for these sheaths to exert was evidently heightened by

FIG. 9. Effect of planting sucker in inclined position under conditions which delay geotropic curvature. Sucker planted at angle of 45° on Dec. 11, 1928, photographed Jan. 30, 1929. The old false-stem is shown at right, the new, formed by the young leaves bursting through base of former, has assumed an upright position.

the long period of suspended growth they had experienced, during which their tissues became more firm than is normal for the sheaths of immature leaves. The new false-stem grew rapidly, and soon formed a thriving young plant arising from the base of the old, decaying false-stem (figs. 9 and 10).

By March 27, 12 of the 15 plants had burst through the base of the old false-stem. By April 11, 13 had burst through the base and 1 had burst through the side near the top, a not infrequent occurrence in sword suckers set erect in replacing lost plants in established plantations.

After the new, upright false-stem had been formed, the growing point formed a proliferation from the old bulb, and gradually curved into an erect position (fig. 10).

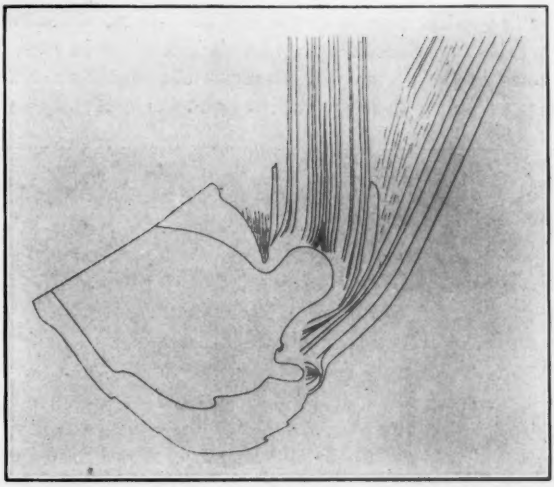

FIG. 10. Section through base of plant similar to one shown in fig. 9. Old false-stem at right; bulb severed from parent rhizome at left. (× ¼.)

V. Reactions to darkness

REGULATION OF THE LENGTH OF THE "INTERNODES"

The regulation of the length of the leaf-sheaths is of the utmost importance to the plant. If for any reason a sheath should remain shorter than that which immediately precedes it, the lamina would not be carried free of the false-stem, could not unfurl, and the symmetry of the plant would be destroyed. In plants of banana and abacá afflicted with "bunchy top," a disease caused by a filterable virus (see OCFEMIA, **4**), successive sheaths do become somewhat shorter, with the result that the leaves are crowded into an unsightly rosette at the top of the false-stem, instead of being distributed along a gracefully tapering trunk. When a sucker is transplanted without having been cut back, the leaves which would have been the next to unfold fail to emerge normally, and remain partially or completely enclosed in the false-stem, while the plant is always put to more or less inconvenience by this derangement of its growth.

It is apparent from anatomical studies that the "normal" length of the sheath is determined by inner causes at the growing point, which produces rudiments of leaves which have progressively longer sheaths, just as they

have larger laminae, until after the stem has begun to elongate rapidly and push upward through the center of the false-stem. The leaves borne on the upper portion of the fruiting stem are separated by long internodes and possess sheaths which fall off rapidly in length, since the point of their insertion is much nearer the top of the false-stem, and it is not necessary that they be so long. The laminae of these leaves also decrease rapidly in size (see 6, table I).

We call the columnar trunk of a banana plant a false-stem. By analogy, we may term the region where each petiole separates from the false-stem a "false-node," and the interval between successive false-nodes may be called "false-internodes." The false-node is not a very definite point, for the upper extremity of the sheath only gradually narrows into a petiole, and it is impossible to set a distinct boundary between the two. Accordingly, in determining the length of the false-internodes, it is best to measure from the point where the descending petiole first touches the false-stem. Since, in the young plants, the sheaths are inserted almost on a level on the abbreviated rhizome, the length of the false-internode is largely an expression of the difference between the lengths of successive sheaths. Thus, if an internode becomes longer than normal, it means that a sheath, for some reason or other, has become abnormally lengthened. Changes in the length of the false-internodes are the best measure of the influence of any condition on the length of the leaf-sheaths.

Various attempts were made to control the final length attained by the sheaths, for although limits are set to this length by the formative processes which apparently terminate long before the leaf makes its appearance at the top of the false-stem, it seemed probable that the degree of elongation of the organ could be altered, to a certain extent, by experimental means. Cutting off the top of the false-stem, so that the leaf emerges and expands sooner than normal, considerably decreases the length of the sheath, probably as a result of the earlier exposure of the lamina to light and transpiration. Cutting off the precursory appendage when it first appears from the top of the false-stem was found to have no effect on the length of the sheath. Binding the lamina with cord, as it emerged from the top of the false-stem, to prevent its unrolling, likewise had no appreciable effect on the length of the sheath. The idea that the expansion of the lamina might be the signal for the gradual cessation of elongation (see section II) therefore proved erroneous. The removal of the lamina bit by bit as it emerged likewise had no significant effect on the length of its sheath.

One's casual experience on the plantation suggests that the intensity of light is of some influence in determining the length of the false-internodes. In young suckers growing up in the shade of dense, unpruned banana groves, these internodes are very long; in shoots arising from propagating

material set out in the open, and therefore exposed to full sunlight, they are very short. Since these two types of shoots differ greatly in other important morphological respects, the conclusion that light was the controlling factor in this case could not be accepted without experimental confirmation. There is also great variation in the normal length of the false-internodes in different varieties of *Musa sapientum*. Thus, plants of the "Martini" type (subspecies *seminifera*) normally have longer false-internodes than the edible "Lady Finger" banana.

Preliminary experiments showed that young banana suckers growing in continuous darkness produce false-internodes of surprisingly great length. In one experiment a young sucker 153 cm. high produced a false-internode 43 cm. in length, while in normal suckers of the same height and variety growing in the same field, the upper internodes were 10–20 cm. in length.

Four portable houses of the type shown in figure 11 were used to study the behavior of banana plants in total darkness. Built of 1 inch boards and covered with a corrugated iron roof, they measured 90 cm. square by 210 cm. high at the front (3 x 3 x 7 ft.). Each was equipped with two venti-

FIG. 11. Three of the dark houses used in experiments, set up in field.

lators, one at the top and one at the bottom, consisting of rectangular boxes open at the ends and crossed by a series of baffle plates which, when blackened on the inside, completely excluded the light but allowed the free passage of air. They were without floors, and the door occupied almost the entire width of the front, to allow them to be easily slipped over the growing plants. The houses were lined with black paper so as to be com-

pletely light-proof, and during the course of the experiments were draped with white sheets to prevent the interior becoming too warm in the sunlight. The highest temperature recorded in them on sunny afternoons was 35° C. They were equipped with handles and shifted about the field as desired by a crew of six men.

The experiments were all performed with various undescribed varieties of the subspecies *seminifera*. Although introduced to Panama from the Philippine Islands under different native names, all showed great similarity in external characters. To start an experiment, I selected a mat (*i.e.*, a clump of plants derived from the branching of a single individual) which contained two young suckers, already producing broad leaves, of very nearly the same height, and if possible with the two youngest visible leaves in approximately the same stage of development. The suckers were 80 cm. or less high, to allow plenty of head room in the dark chamber, and close enough together to go in easily through the door. These conditions were not always easy to fulfil, and many mats were examined before a satisfactory one was found. Except for these two suckers, all the other plants in the mat were pruned away at the ground level, and their growing points destroyed. Thus the experimental plants had the benefit of the entire root system and the large food reserves of the parent rhizome (see fig. 12). All of the laminae were then cut away from one member of the pair, while the other was left intact (see fig. 13). The plants were measured and marked so that further growth could be determined; the dark house was then moved over them, banked with earth to exclude light at the bottom, draped with sheets, and the experiment begun. Thereafter it was opened only at night, when the portion of the lamina of the defoliated plant which had emerged during the last 24 hours was cut away.[1]

The duration of the experiments was limited by the height of the houses. In every case, however, the plants remained in darkness long enough for the complete expansion of at least one leaf beyond that in the process of emerging from the false-stem at the beginning of the experiment. In most cases time was allowed for two and in many instances three leaves to perform the entire process of emergence in total darkness. Thus, in every plant at least one new false-internode was added to the false-stem during the period in darkness. The more important data collected in these experiments are summarized in table III. Here both plants of each pair bear the

[1] The objection might be raised that the removal of the false-stem and growing point of the parent plant causes the suckers to produce longer internodes, just as it causes them to produce broader laminae. This, however, is not the case with plants growing in the light. In a few experiments, suckers still attached to the uninjured parent stems were enclosed in lightproof cylinders constructed of wire and black paper, and these behaved in all respects like the plants described in the text.

FIG. 12. A pair of plants prepared for an experiment (with the exception of removing laminae from one), showing type of suckers used.

FIG. 13. Plants 3, A and B (see table III), at conclusion of the experiment. The 12 leaves bear the white tags. April 15, 1929.

TABLE III

GROWTH OF BANANA SUCKERS IN DARKNESS

(EXPERIMENTS EXTENDED FROM MARCH 22 TO MAY 30, 1929, ONLY FOUR NUMBERS COULD BE PERFORMED SIMULTANEOUSLY)

No.	PERIOD	ORIGINAL HEIGHT		FINAL HEIGHT		GROWTH		LENGTH OF FALSE INTERNODES							
								−2		−1		+1		+2	
		A[1]	B[2]	A	B	A	B	A	B	A	B	A	B	A	B
	days	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	24	70	70	125	120	55	50	14	11.5	24	17	34	23.5	0	8
2	19	78	73	142	112	64	39	17	13	20	18	32	10	19	14
3	24	60	65	115	93	55	28	15	14.5	19.5	12.5	33	14.5	0	0
4	19	76	80	123	107	47	27	7.5	9.5	16	17	38.5	13	0	0
5	24	37	41	130	102	93	61	16.5	20	24	17.5	26.5	18	11.5	8.5
6	22	66	67	135	103	69	36	17.5	18	22	18	23.5	11	19	7
7	22	60	63	128	95	68	32	11.5	12	17	9	32	11	25	13
8	22	67	83	128	130	61	47	16.5	16.5	25	21.5	35	10	0	1
9	28	59	65	140	116	81	51	12.5	17	19	17	30	20	29	9.5
10	28	47	56	107	136	60	80	15.5	19.5	29.5	21.5	28.5	22	0	22.5
11	21	78	79	135	112	57	33		17.5	16	21	32.5	22	30	18
Average						64.5	44.	14.4	15.4	21.7	17.3	31.4	15.9		

[1] A = normal plant.

[2] B = defoliated plant.

same number, the "normal" plant is designated by "A," the plant with the laminae removed by "B." The data for the A and B plants of each pair are arranged in alternating columns to facilitate comparison. The leaf partially emerged at the beginning of the experiment is designated by the number + 1, and the following leaves are numbered + 2, + 3, etc. The leaves completely emerged at the commencement of the experiment are designated by a minus sign before the number. Leaf − 1 is the youngest of these, − 2 the next in age, etc. The false-internodes are designated by the numbers of the leaves which terminate them.

We will consider first the data for the A plants. With a single exception, the longest false-internode produced by the plant was terminated by the lamina which was actually emerging when the plants were placed in darkness, indicating that the sheath belonging to this lamina was the first whose growth was markedly stimulated by the absence of light. The sheath of the next-older leaf—the youngest completely expanded when the experiment began—was also stimulated to increased growth as a result of the treatment. This is indicated by the abnormal length, in a number of plants, of the internodes terminated by these leaves, and by the marked increase (almost 7 cm.) of the average length of these internodes over those below them. Although the sheaths of 5 or 6 of these older leaves continued to grow at a rate inverse to their age, their final length was not greatly affected by the absence of light . In suckers of the size used in the experiments, growing in the light, a slight growth may sometimes be detected in even the seventh or eighth leaf from the top.

The length of the sheath of leaf + 2 is still more greatly exaggerated (beyond what it may have been in the light) than that of leaf + 1. This fact is masked, in the manner of presentation of the data we are now employing, by the abnormal length of internode + 1, and it must be remembered that the effects are additive in successive internodes. It was not possible, for lack of space, to allow these sheaths to approach their maximum length, but already several of them have formed internodes well above the maximum for plants growing in light. The length of the sheath of the youngest expanded leaf of each plant at the conclusion of the experiment is, within a few centimeters, the same as the final height of the false-stem as given in the fifth column of the table.

Suckers of the same variety and height as those used in the experiments, growing in the same field but under the shade of the older plants of the mat, rarely show internodes over 20 cm. in length. The maximum length recorded in 15 such plants chosen at random was 22 cm. Most of the upper internodes of such plants fall between 8–20 cm. in length. If the suckers are exposed to strong sunlight by cutting away the older plants, the false-internodes become much shorter. The shortest "+ 1" internode formed by

the experimental plants measured 23.5 cm., while the average of 31.4 cm. is almost 50 per cent. above the maximum for plants growing in the light. Just as in plants with elongated stems etiolation causes the production of lengthened internodes, so in the false-stem of the banana darkness causes the formation of greatly exaggerated false-internodes. This is a result of the abnormal lengthening of the sheaths of the leaves which emerge in the dark. No change in the configuration of the rhizome was observed in the experimental plants.

Location of the increased growth caused by darkness

The average separation of the transverse septa in the various regions of the leaf-sheaths of a plant growing in the open is shown graphically in figure 14 (at left), where the distance occupied by each 50 septa is marked off on a vertical line drawn in proportion to the length of the sheath. Here

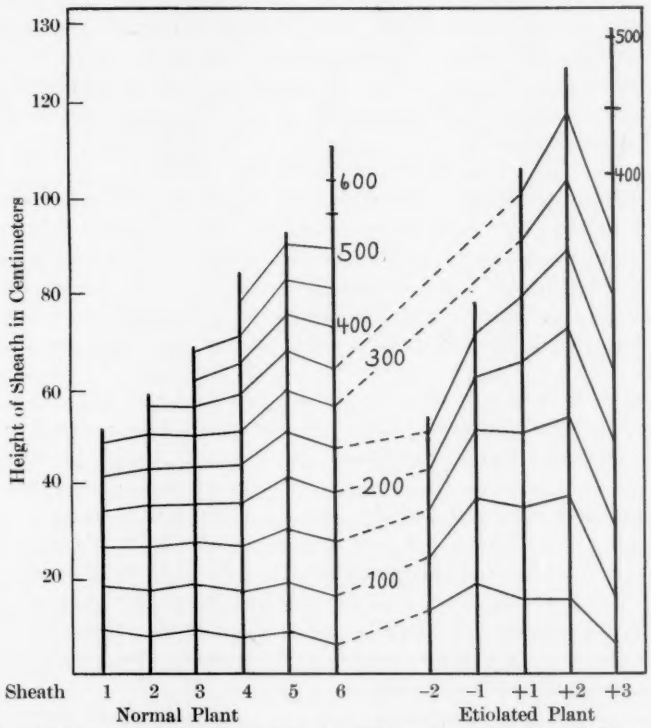

Fig. 14. Distribution of transverse septa in sheaths of normal plant (left) and plant which had been in darkness for 42 days, during which false-stem grew from 70 to 141 cm.

it will be seen that the septa are farthest apart at the base of the sheath, and gradually become more crowded toward the apex. Each figure employed in the construction of the graph represents the average of the counts of four parallel series of septa running the length of the sheath, and all from the central part. There is usually a regular increase in the number of septa in successively older sheaths. In sheath 5, however, for some reason no more septa were formed than in the preceding sheath, although the sheath itself is 9 cm. longer. As a result, the septa in all portions of sheath 5, but especially in the basal region, were somewhat more distant than in the older sheaths, indicating that the plant is able to compensate a deficiency in the formative processes by an abnormal amount of elongation at a later stage.

At the right of the figure a similar graph is presented for a plant which was kept in the dark house for 42 days, during which the height of the false-stem increased from 70 to 141 cm., and 3 new leaves (+1, +2, and +3) expanded. Leaf −2 was affected to a certain extent by the treatment, and the increased elongation occurred chiefly in the basal portion of the sheath (first 100 septa). In leaf −1 the increased length is accounted for principally in the region of the basal 150 septa. The separation of septa in this sheath is exaggerated by a deficiency in their number, for it contains no more than the preceding one. In the following sheath (+1) the separation of septa is greater than normal in all regions, but the excess growth is most marked in the basal half. In sheath +2 there is a marked increase in the separation of the septa throughout the sheath, including its apical portions. In the sheath of the latest leaf to emerge (+3) the separation of the septa in the distal portion is still greater. This leaf was still rapidly elongating, and in its less mature basal portion the septa are still quite crowded.

From these data (and other similar counts which lack of space excludes here) it is evident that in leaves already expanded, or just emerging at the time the plant is placed in darkness, the increased growth is localized at the base of the sheath. In successively younger leaves the influence of darkness is felt higher and higher in the sheath, with the result that the apical regions also stretch more strongly, and their transverse septa become far more distant, than in leaves emerging in the light. It is because of the more extensive region affected in the younger leaves that they are able to maintain the exaggerated length of the false-internodes begun by the first leaves to emerge in darkness, which in each succeeding sheath means a proportionally greater stretching.

Localization of perception

The intercalary growing zone at the base of the sheath of the banana leaf is covered by a number of other leaves which more or less effectively

screen it from the light. Hence it becomes of interest to determine whether the increased growth of the sheath in darkness occurs because the sheath itself fails to receive the very small amount of light it normally may receive, or as a result of changes induced by continued darkness in the lamina and thence transmitted to the growing sheath.

Observations with a diaphanoscope revealed that a certain amount of light penetrates to the base of the sheath of the emerging leaf in plants of the size employed in the experiments, although this sheath can at best enjoy a very dim illumination, most of the rays being absorbed or reflected by the 5 or 6 older sheaths through which they must filter. None-the-less, it was not *a priori* certain that the absence of this weak illumination did not cause the observed changes. To test this point, the laminae of one plant of each pair employed in the dark chamber experiments were removed as described above. The performance of these plants is recorded in table III, in the columns headed "B." Here it is seen that the plants without laminae formed internodes not longer than those commonly found in plants growing in the open. The average length of the +1 internodes of the defoliated plants is half that of the A plants, and the maximum for the defoliated plants is the same as the minimum for the normal plants. Because the false-stems of the defoliated plants remained shorter, the leaves often emerged in more rapid succession than in the normal plant of the pair, hence the earlier formation of the +2 internode observed in some cases.

In order to determine whether the removal of the laminae had in itself any effect upon the formation of the subsequent leaves or the length of their sheaths, plants growing in the open were defoliated in the same manner as the B plants in the dark houses. The newly emerging leaves of three of these plants were cut off daily for seven weeks, during which they formed 5–7 new internodes of the usual length. These plants grew in the shade of the older plants of the mat.

As a control, the false-stems of 10 suckers, most of them somewhat larger than those used in the dark houses, were wrapped in several thicknesses of heavy, black mulch paper, wound around them in a spiral like a roller bandage. By this means they were effectively darkened. The paper was covered on the outside by white cloth, to prevent its becoming heated in the sunlight. It was necessary to rewrap the stems at intervals of several days, as with increasing girth they began to press against their wrappings, and to enclose the newly formed portions. The experiment was continued 24–25 days, slightly longer than most of the dark chamber experiments. The longest internode formed by these plants measured 26.5 cm., somewhat longer than normal, but 5 cm. below the average of the +1 internodes of the A plants in the dark chamber experiments (see table IV).

TABLE IV

LENGTH OF FALSE-INTERNODES FORMED BY PLANTS WITH FALSE-STEMS DARKENED

No.	DATE BEGUN	DURATION	ORIGINAL HEIGHT	FINAL HEIGHT	LENGTH OF FALSE-INTERNODES		
					−2	−1	+1
		days	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	3–25–29	25	102	146	23.5	16	19.5
2	"	25	85	132	19.5	19	18.0
3	"	25	112	156	19.5	16	13.0
4	"	25	112	164	22.5	20.5	18.5
5	4–12–29	24	78	133	20.5	25.5	18.5
6	"	24	83	139	18.0	23.5	19.5
7	"	24	92	167	21.0	25	26.5
8	"	24	80	119	15.5	15	12.5
9	"	24	78	117	19.0	19	16.0
10	"	24	89	135	16.0	19	14.0

From these experiments it seems that the abnormal lengthening of the sheaths of plants growing in darkness is largely a result of some influence which their laminae exert upon them, although a slight direct effect of darkening on the sheaths themselves is not precluded (*cf.* table IV). This influence is transmitted to the basal growing zone of the emerging leaf, and causes an abnormal amount of elongation there, as indicated by the increased separation of the transverse septa. The influence of the leaves already in the dark chamber is likewise transmitted to the sheaths of younger leaves which have not yet made their appearance, and causes increased elongation in all portions, including regions which, at the time of the emergence of their own lamina, are too mature to be appreciably influenced directly by it.

The literature on plant hormones has already become a most extensive one, and a number of complicated phenomena of transmitted influences in the plant body seem most readily explained by the presence of such substances. WENT (**10**) found that a growth-promoting substance, formed in the tip of the coleoptile of *Avena* seedlings, is transmitted to the more basal portions of the organ and causes accelerated growth there. He determined that the growth-promoting substance of the coleoptile tip is formed more abundantly in darkness than in light. It is suggested that the phenomena described in this section may be caused by the production of a growth-promoting substance in the lamina of the banana leaf in darkness. This substance, carried basally in the phloem, may cause an abnormal degree of elongation of the tissues in the lower part of the sheath. It may also produce increased growth in the sheaths of younger leaves whose laminae are

still enclosed in the false-stem, and accordingly in absolute darkness are receiving only slightly less light than in a plant growing in the open. If the great distance (1 meter or more) between the organ of perception and the region of response is urged against this view, it must be remembered that the phloem in the vascular bundles in the leaf-sheath is extremely well developed (**6**, fig. 18) and translocation very rapid. In the fruiting banana plant, the carbohydrate formed in most of the leaves must be carried all the way down to the bulb in the leaf-sheaths, and thence all the way up the stem to the bunch of developing fruit, a total distance, in large plants of 12–14 m.

Effect of etiolation on size of lamina

Continued darkness does not cause a reduction in the size of the lamina. The leaves which expanded in the dark chambers were in all cases longer and usually broader than those which preceded them, as is normal in immature banana plants. During the course of several of the experiments the lateral buds on the old rhizomes developed into vigorous young suckers, sometimes reaching over 1 m. in height of false-stem, and produced leaves of the dimensions usual for suckers of their size.

MacDougal (**2**), who studied the behavior of a large number of species in darkness, found that many monocotyledonous leaves with parallel venation, arising directly from an underground tuber, bulb or rhizome, attain in darkness a length and sometimes a breadth in excess of the normal, and an area equal to or greater than the normal organ. Where there is a distinct petiole, or sheath, arising from near the ground level, these are usually excessively elongated. *Canna* agrees with *Musa* in the production of abnormally long sheaths in darkness. The laminae of many monocotyledonous leaves (especially Araceae) with distinct petioles or sheaths are considerably smaller than normal, and often fail to unroll completely, in this respect differing decidedly from the banana.

VI. Conclusions

A dicotyledonous plant with elongated internodes, a coleus or sunflower, for example, exhibits certain responses by which it adjusts itself when its stem is placed in a horizontal position, or its light is seriously reduced. The banana is structurally very different from these plants and although the morphological nature of the organs by which the reactions are accomplished is different, its responses to the same conditions are surprisingly similar. A sunflower reacts to darkness by increasing the length of its internodes, a banana by increasing the length of its leaf-sheaths, which results in the production of longer false-internodes. Placed on its side, the dicotyledonous plant erects itself by the upward curvature of its stem, the

banana by the upward curvature of the false-stem made up of leaf-sheaths, aided to a certain extent by the bulb. In this case the banana's method seems the more efficient, since it results in the erection of the entire false-stem from the base, not merely of the younger internodes, as is the case with the sunflower. Banana plants are able to erect themselves in this manner after they acquire a height and weight which would preclude such a response on the part of most plants. Far from being rendered relatively rigid and unresponsive by its method of enclosing the growing point within massive sheaths, the banana possesses an organization which is remarkably plastic and adjustable. It must be remembered that it is essentially a plant of quiet places; violent windstorms are disastrous to it.

In conclusion, it is a pleasure to acknowledge my gratitude to Dr. JOHN R. JOHNSTON, Director of Agricultural Research of the United Fruit Company of Boston, for extending to me the use of the Company's research stations in Central America, and to Mr. JOSEPH H. PERMAR, in charge of the station near Almirante, Panama, for his unfailing cooperation and interest, without which it would have been impossible to carry out much of the work reported here. To him I am also indebted for the communication of many valuable observations, the result of long familiarity with the banana plant. To Professor DUNCAN S. JOHNSON, of Johns Hopkins University, I owe a debt of gratitude for the interest and encouragement he has continued to show in my work.

Summary

1. Great pressure is required to force the emerging leaf of the banana upward through the false-stem. If a section is removed from the side of the false-stem, depriving the sheath of this leaf of its lateral support, it is unable to transmit the pressure exerted by the basal growing zone. The leaf accordingly is not able to emerge, and subsequent leaves break out through the aperture, forming a forked false-stem.

2. When the pressure on the basal growing zone is reduced by cutting off the top of the false-stem, the rate of elongation is immediately increased 5–9 times, but soon falls to its original value.

3. Seedlings placed in a horizontal position erect themselves by a curvature of the base of the false-stem.

4. Plants with false-stems up to 2 m. in height, 20 cm. in basal diameter, and a total weight of aerial portions up to 15 kg., erect themselves from the base when inclined at an angle of 45° or less with the ground.

5. The erection of these plants is accomplished principally by the curvature of the false-stem at its base. The sheaths on the lower side become thicker as a result of the enlargement of their cells without division. There is a decrease of starch on this side.

6. The cortical cells on the lower side of the rhizome elongate parallel to the long axis of the plant and divide by one or more transverse walls. The cell walls become thinner, and the starch grains they contained largely disappear. The whole cortex becomes thicker on the lower side than on the upper. As a result, the false-stem is pushed upward.

7. When a sucker is dug up and replanted in an inclined position, growth of the top is suspended for about a month, while a root system becomes established. Upon renewal of growth, the old false-stem is unable to erect itself. The young leaves, imprisoned within it, burst through the upper side of the old false-stem, at the base, and establish a new false-stem in an upright position.

8. When the plant is placed in darkness, the leaf-sheaths become abnormally long, with the result that false-internodes of about twice the normal length are formed.

9. Sheaths from which the laminae have been removed do not become abnormally long in the dark, although in the open the removal of the lamina does not affect the length of the sheath. When the false-stems are darkened but the laminae remain in the light, the sheaths become only slightly longer than in plants growing in the open.

10. Laminae kept in continuous darkness exert some influence on the basal growing region of their sheaths, about a meter distant, which results in their increased elongation. It seems likely that a hormone is responsible for this effect.

11. There is no reduction in the size of the lamina of plants grown in darkness.

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RELATION OF H-ION CONCENTRATION OF TISSUE FLUIDS TO THE DISTRIBUTION OF IRON IN PLANTS

R. A. Ingalls and J. W. Shive

(WITH TEN FIGURES)

Although iron is an essential element for plant growth, it is not always present in an available form for assimilation by the plant. Factors influencing the availability of iron are not well understood. Previous investigations of the iron problem have dealt mainly with a study of the external medium in which the plants were grown, with less emphasis attached to the study of the conditions existing within the plants themselves.

Patten and Main (**18**) found that iron was precipitated from solution in varying degrees from pH 3.5 to 6.0, practically all being precipitated at 6.0 and above, thus rendering it unavailable for absorption by the plant. This was also brought out by Hopkins and Wann (**10**). They found difficulty in growing plants in a medium of pH 6.0, due to the fact that iron was removed by adsorption on calcium phosphate which gradually precipitated as the solution became alkaline, a physical chemical effect capable of influencing iron availability within the plant as well as in culture media.

The fact that lack of available iron is not entirely due to the conditions existing in the culture media may be shown by reference to the work of Appel (**2**). He found that buckwheat plants were less sensitive to changes in reaction of culture media than were corn plants, corn requiring much more iron than buckwheat. Little difficulty, therefore, was experienced in growing buckwheat without chlorosis in solution cultures in which corn suffered from lack of available iron. After a study of the internal conditions existing within the plant, presented in the following pages, an explanation of this phenomenon may be attempted.

Loehwing (**13**) has reported that plants grown in a medium high in lime display peculiar iron immobility characterized by copious precipitation in the roots. He states that the lime reduces the sap acidity to the point of interference with internal iron mobility. Lime injury involving chlorosis has been reported for corn by Mazé (**16**), for pineapples by Gile (**5**), for rice by Gile and Carrero (**6**), for pears by Milad (**17**), and for citrus fruits by Lipman (**22**).

In addition to the work cited, some work has been reported on the H-ion concentration of tissue fluids. Haas (**8**) made studies of actual and total acidities and of the total alkalinity of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon

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Loehwing (**13**) has reported that plants grown in a medium high in lime display peculiar iron immobility characterized by copious precipitation in the roots. He states that the lime reduces the sap acidity to the point of interference with internal iron mobility. Lime injury involving chlorosis has been reported for corn by Mazé (**16**), for pineapples by Gile (**5**), for rice by Gile and Carrero (**6**), for pears by Milad (**17**), and for citrus fruits by Lipman (**22**).

In addition to the work cited, some work has been reported on the H-ion concentration of tissue fluids. Haas (**8**) made studies of actual and total acidities and of the total alkalinity of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon

these acidities. In this connection he reports that in ten out of fourteen cases the addition of lime was followed by a decrease in actual acidity of the plant juice, which seems to point to the fact that plant juices are influenced to some degree by the nature of the medium in which they are grown. Other points of interest in his study of plant juices were as follows: (1) the presence of a hydrogen-ion concentration gradient for tissue fluids which is not always in the same direction in different species or in the stems and leaves of the same species; (2) an increase in acidity with age increase in the plant, and (3) that illumination tends to decrease the acidity of the plant. This point was further brought out by GUSTAFSON (**7**). He worked with *Bryophyllum calycinum*, determining the pH of its juice at various intervals throughout the day and the early part of the evening. He found that the acidity of the juice decreased during the day and increased again at night, but if the plant was kept in the dark continuously for 24 hours or more, the acidity did not continue to increase but began gradually to decrease, as the food supply was used up in respiration. The highest acidity was reached at 10 A. M. while the lowest was obtained at 4 P. M. The experiment was carried out during both clear and cloudy days, and it was found that the same general results were obtained on both types of days but to a different degree.

CLEVENGER (**4**) found a similar nocturnal change in pH of the tissue extract of cowpeas due to change in light intensity. He also pointed out that the pH varied with temperature, being higher at high temperatures than at low temperatures. ATKINS (**3**) made a study of the variation in the juices of different plants and found a range in pH from 1.4 to 8.0, but only in rare cases did he find any above 7.0.

The purpose of this investigation has been to determine first, the influence of day to night variations in light intensity upon the hydrogen-ion concentration of the plant tissue fluids of a number of different species; second, to study the relation between hydrogen-ion concentration of the plant tissue fluids, as influenced by variation in light intensity, and the "filterable" or soluble iron content of the plant tissues; third, to study the relation between hydrogen-ion concentration of the plant tissue fluids and the total iron content of the plant tissues; and finally, to study the influence of these internal factors upon the iron mobility, its distribution, and ability to function in the plant tissues.

Experimental methods

Plants used in the first part of this investigation were grown in sand and solution cultures to insure as uniform a medium of growth as possible. It was later found, however, that quite similar results were obtained with

plants grown in soil plots, provided that care was taken to select plants of the same age which had been grown under similar conditions. The species here used may be classed into two groups according to the purpose for which they were grown. Group 1 comprised buckwheat (*Fagopyrum esculentum*), clover (*Trifolium repens*), sedum (*Sedum reflexum*), and bryophyllum (*Bryophyllum calycinum*). These were grown for the express purpose of studying the influence of light intensity on the pH of the tissue fluids. The hydrogen-ion concentration of the juices of both stems and leaves of the plants of this group were determined at intervals of two hours during the day and night. Care was taken to select the various experimental periods at times when the sky was cloudless during the day in order that the plants might be subjected to the maximum variation in light intensity during a day and night period.

Group 2 comprised buckwheat (*Fagopyrum esculentum*), bryophyllum (*Bryophyllum calycinum*), rumex (*Rumex patientia*), sedum (*Sedum reflexum*), tobacco (*Nicotiana rustica*) *tomato* (*Lycopersicum esculentum*), asparagus (*Asparagus officinalis*), soybean (*Glycine max*), and clover (*Trifolium repens*), and were grown for the purpose of determining the filterable or soluble iron content of tissue fluids of plants having different pH values, and also to determine the filterable iron content of the tissue fluids of plants of the same species at various intervals throughout a day and night period.

Sand cultures were grown in clay percolators similar to those described by ALLISON AND SHIVE (**1**). They were approximately 31 cm. in height with a diameter of 15 cm. at the top and 5 cm. at the base. The opening through the bottom of each percolator was closed with a cork carrying a short glass tube through which the percolating solution could escape. The upper end of the tube was closed by a small plug of glass wool. Each percolator contained about 5 kg. of quartz sand which had been thoroughly washed in an agate pan until the overflowing water was free from sediment. TOTTINGHAM'S (**25**) solution modified by JONES and SHIVE (**11**) was supplied to each culture. This solution was continuously renewed by means of a drip and drain system (**22**) which allowed one liter of new solution to flow into each culture at a constant rate during a period of 24 hours. Each culture was flushed once a week with distilled water to avoid any excessive salt concentration which might have resulted from evaporation at the surface of the sand, or from water loss through transpiration. Buckwheat and soybeans were grown in solution cultures in two-quart colorless glass jars. They also were supplied with the constant solution renewal system described by SHIVE and STAHL (**22**). In both sand and solution

cultures, iron was supplied to the plants in the form of a freshly prepared ferrous sulphate solution containing 0.1 mg. of iron per cc. of solution.

In the early stages of growth, 0.1 cc. of the iron solution containing 0.1 mg. of iron per cc. was added to 1000 cc. of nutrient solution. This amount, however, was increased and sometimes reduced during the later stages of growth, as the external conditions and the requirements of the plants made this necessary.

Seeds used in these experiments were germinated between moist filter-papers and then transferred to a germinating net as described by SHIVE (**21**) When the seedlings were 4 cm. tall they were carefully selected for uniformity of size and vigor and transferred to their respective media, ten plants being used in each sand culture and three in each solution culture.

To prepare the material for the extraction of the tissue, it was cut into small pieces and placed in test tubes. These were then plugged with paraffined cork stoppers plunged into a salt-ice mixture and frozen as quickly as possible in order to prevent any appreciable chemical change before freezing. In all cases duplicate samples were used. Preparatory to expressing the tissue fluids, the test tubes containing the samples were placed in tepid water and the material allowed to thaw. This usually required from five to ten minutes. At this stage, the material was removed from the tubes and the juice extracted by means of a small screw press. Precautions were taken to prevent the tissue fluids from coming in contact with anything except glass surfaces.

pH determinations were made electrometrically by means of the hydrogen electrode and a type K Leeds and Northrup potentiometer. About 5 cc. of juice were used at each determination. It was placed in a short Pyrex tube and hydrogen was allowed to bubble through until a constant potential was attained. Electrodes were cleaned and platinized before making determinations and again frequently throughout the experiments. They were also checked against a standard acetate solution of known pH value.

Samples of green plant tissues of the various species studied, from which the moisture content and total iron content of the plant were determined, were taken at the end of each experimental interval (usually at the end of two hour intervals) throughout a day and night period. For total iron determinations, the plant tissues were dried in an oven at about 85° C. for 48 hours and at 100° C.–102° C. for 24 hours. They were then ground to a powder with a pestle and mortar in order that uniform samples might be obtained. The material was then placed in a desiccator over night, after which duplicate samples of 0.1 gm. each were weighed out and placed in Pyrex test tubes. Iron analyses were made according to a method described

by WONG (**26**). It consisted in completely digesting the weighed sample with 1 cc. of concentrated iron-free sulphuric acid, allowing the contents of the tube to cool for about 20 seconds and then adding to it a ten per cent. solution of sodium chlorate. This solution was added carefully drop by drop and allowed to run down the side of the tube to prevent excess spattering when the solutions came in contact with one another. Boiling was then continued until white fumes appeared. The content of the tube was clear and colorless when oxidation was completed.

Potassium sulphocyanate, 5 cc. per sample, was used as an indicator, and the contents made to a known volume with distilled water. The red color produced by the indicator varied in depth according to the amount of iron present. Each sample containing the unknown was compared in a Dubosecq colorimeter against a standard iron solution. This solution was prepared by dissolving 0.7 gm. of recrystallized ferrous ammonium sulphate in about 50 cc. of distilled water. To this was added 20 cc. of 10 per cent. sulphuric acid and then sufficient one-tenth normal potassium permanganate solution was added to just oxidize the ferrous salt completely. It was then diluted to 1 liter. This solution contained 0.1 mg. of iron per cc.

To both the standard and the unknown iron solution to be determined was added 0.25 cc. of dilute (30 per cent.) nitric acid. It was found that the addition of this solution prevented the reduction of iron from the ferric to the ferrous form, thus preserving the color of the test and standard solutions without change for a sufficient length of time to compare with accuracy at least twelve unknowns against the same sample of the standard.

Since it was impossible to remove all the moisture by pressure from the plant tissue, some means had to be adopted by which total filterable iron could be determined on the basis of dry plant tissue. This was accomplished as follows:

The tissue for which iron determinations were to be made was cut into rather small pieces and thoroughly mixed. One sample was taken from the mixed tissue and frozen in order to extract the tissue fluid. Iron analyses were made on 1 cc. of the extracted filtered fluid according to the method previously described. The remainder of the plant tissue was weighed, dried, and the moisture content determined. From this, the amount of moisture per gram of dried plant tissue was calculated.

The quantitative iron analyses on 1 cc. samples of the filtered tissue fluid, together with the determinations of moisture and the content of solid material in the filtrates, furnished the necessary basis for the calculation of the iron per gram of dry material in the plant tissues in question.

Experimental results

Introductory

The practice of supplying iron to certain species grown in artificial media in suitable proportions and in such a manner as to prevent the appearance of chlorosis from lack of this element and produce healthy green plants is attended with considerable difficulty. To accomplish this requires a knowledge of the internal nature of the particular species in question as well as the chemical nature of the culture media.

It has long been observed that not only do plants of different species require different amounts of iron, but also that plants of the same species require different amounts of iron from time to time, depending upon the degree of light intensity: that is, the iron requirement of the plants varies with the light intensity. However, this is much more pronounced in some species than in others. Among the species exhibiting the characteristic of marked variation in iron requirement with variation in light intensity are those in which the pH of the tissue fluids lies close to or above the precipitation point of iron; and such plants are frequently employed in experimental work. In this type of plant, chlorosis may occur under certain conditions from lack of iron in the chlorophyllous cells with an adequate concentration of available iron in the culture media. It was found that the proper concentrations of available iron required in the culture medium to prevent chlorosis in these plants during a period of low light intensity, such as a period of cloudy weather, was entirely inadequate to maintain the normal green color in the plants during periods of high light intensity, such as a series of successive clear days during the summer months.

On the other hand, those species in which the pH of the tissue fluids is considerably below the precipitation point of iron do not exhibit any marked variation in the iron requirement with variation in light intensity, nor is the iron concentration in the medium required to maintain the healthy green color in these plants ever so great as it must be for the type of plant in which the pH of the tissue fluids lies near or above the precipitation point of iron. It is thus evident that the cause of chlorosis due to the lack of iron is largely dependent upon factors existing within the plant, through the agency of which the iron may be precipitated at certain points along the paths of translocation in stems or leaves, thus preventing the migration of iron into the chlorophyllous cells.

It is well known that under most field conditions where the soil solution is slightly acid in reaction, plants can usually obtain a sufficient supply of iron during all phases of growth. In certain alkaline soils, however, some important agricultural plants are unable to obtain a sufficient amount of

available iron, thus causing considerable loss in production, while under the same conditions other plants appear to suffer no injury from lack of available iron. It is reasonable to assume, therefore, that an internal mechanism renders small quantities of iron mobile in the plant and available to the chlorophyllous cells. It was with these points in mind that the present investigation was undertaken.

The influence of light intensity on the H-ion concentration of plant tissue fluids

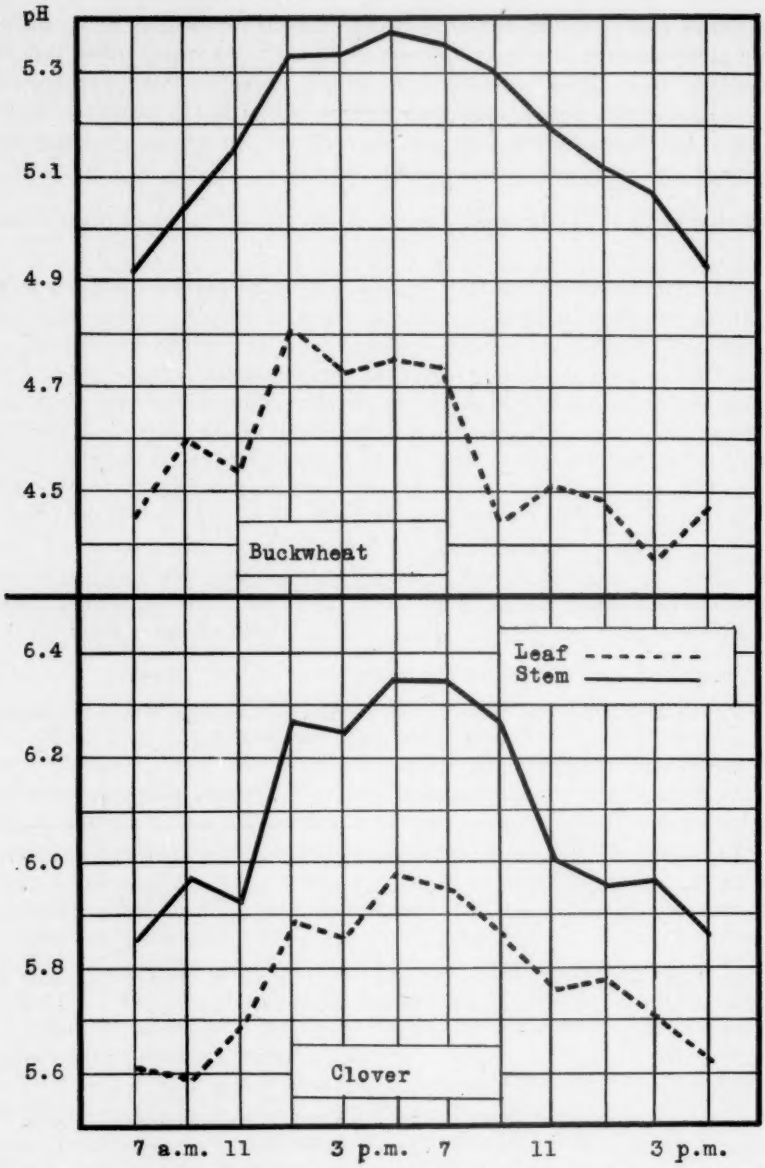
The data obtained from the experiments conducted for the purpose of showing the effect of light intensity on the hydrogen-ion concentration of plant tissue fluids are presented in tables I and II. In the first column of each table is given the time at which pH determinations of tissue fluids were made during a period of 24 hours. In the succeeding columns are given the average pH values of juices of stems and leaves of the plants indicated at the head of the respective columns. Each value represents the average of two or more determinations. The results given in table I are all from plants with thin leaves, while those of table II deal with thick-leaved, fleshy plants.

Figure 1 shows the graphs plotted from the data as given in table I, representing the course of change in the pH values of the stem and leaf juices of buckwheat plants during a period of 24 hours. The experiment from which these data were obtained was carried out in the spring of the year, the

TABLE I

pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF BUCKWHEAT, CLOVER AND *Rumex* PLANTS AT TWO HOUR INTERVALS

TIME	BUCKWHEAT		CLOVER		*Rumex*
	STEMS	LEAVES	STEMS	LEAVES	LEAVES
9:00 A. M.	4.615	5.047	5.596	5.968	4.210
11:00 A. M.	4.531	5.139	5.697	5.934	4.193
1:00 P. M.	4.804	5.342	5.883	6.272	4.328
3:00 P. M.	4.726	5.333	5.868	6,238	4.480
5:00 P. M.	4.767	5.376	5.985	6.342	4.514
7:00 P. M.	4.757	5.351	5.951	6.340	4.311
9:00 P. M.	4.446	5.300	5.866	6.255	4.277
11:00 P. M.	4.548	5.190	5.765	6.002	4.176
1:00 A. M.	4.497	5.139	5.783	5.951	4.193
3:00 A. M.	4.353	5.089	5.714	5.968	4.108
5:00 A. M.	4.447	5.021	5.630	5.850	4.032
7:00 A. M.	4.454	4.920	5.613	5.850	4.057



FIGS. 1 (upper) and 2 (lower). Graphs representing the course of change in pH values of leaf and stem juices of buckwheat and clover plants during a 24-hour experimental interval.

sky being perfectly clear during the experimental interval, so that the plants were exposed to approximately maximum variation of light intensity from day to night for this period of the year. These graphs clearly bring out the fact that there is considerable change in hydrogen-ion concentration of the juices of both stems and leaves which decreases during the day with increase in light intensity and increases during the night. It will be observed that the juices of buckwheat stems are much more acid than are those of the leaves, the stems having a maximum pH of 4.79 and a minimum of 4.45, while the corresponding pH values for leaves are 5.37 and 4.92, respectively. While there is a marked difference in pH values between stems and leaves, as indicated by the graphs, the range of variation in these values over a 24 hour period is about the same for the juices of both stems and leaves. Not only are they alike in this respect, but also there is a pronounced similarity in the general trend of the graphs representing the course of change in pH values. It will be observed, however, that the graph representing pH values of stem juices is somewhat less regular in its general outline than is that representing pH values of leaf juices. Highest H-ion concentration for stems is indicated at 3 A. M. and for leaves at 5 A. M., while minimum H-ion concentrations (maximum pH value) are indicated for stem and leaf tissue fluids at 1 P. M. and 5 P. M., respectively. Although the pH of the stem juices appears to fall after 1 P. M., there is no immediate pronounced decrease until 5 P. M., when the values decrease quite rapidly following the rapid decrease in light intensity.

In figure 2 are given the data for clover as taken from table I and plotted in the same manner as are those for buckwheat in figure 1. The experiment from which these data were obtained was carried out on a clear day similar to that on which the data for buckwheat were obtained. It will be observed that the pH values for the juices of clover are much higher than for those of buckwheat, these values for the juices of clover being above the precipitation point of iron during the day and slightly below this point during the night period. Nevertheless, the range in daily variation appears to be much the same in both species. Here again, the juices of stems are much more acid than are those of the leaves, the stems having a maximum pH value of 5.98 and a minimum of pH 5.59, while the leaves have a maximum of 6.34 and a minimum of 5.83. Highest acidity is indicated at 7:00 A. M. and lowest acidity at 5:00 P. M.

The next plant to be considered is *Sedum*, which is a thick leaved, fleshy succulent. The data for this species are presented in table II and are graphically shown in figure 3. It will be observed that this plant shows the daily change in hydrogen-ion concentration to a much more marked degree than do those already considered. In stems, the range is from pH 4.78 at 7:00 A. M. to 5.49 at 7:00 P. M., while the leaves show a corresponding range

TABLE II

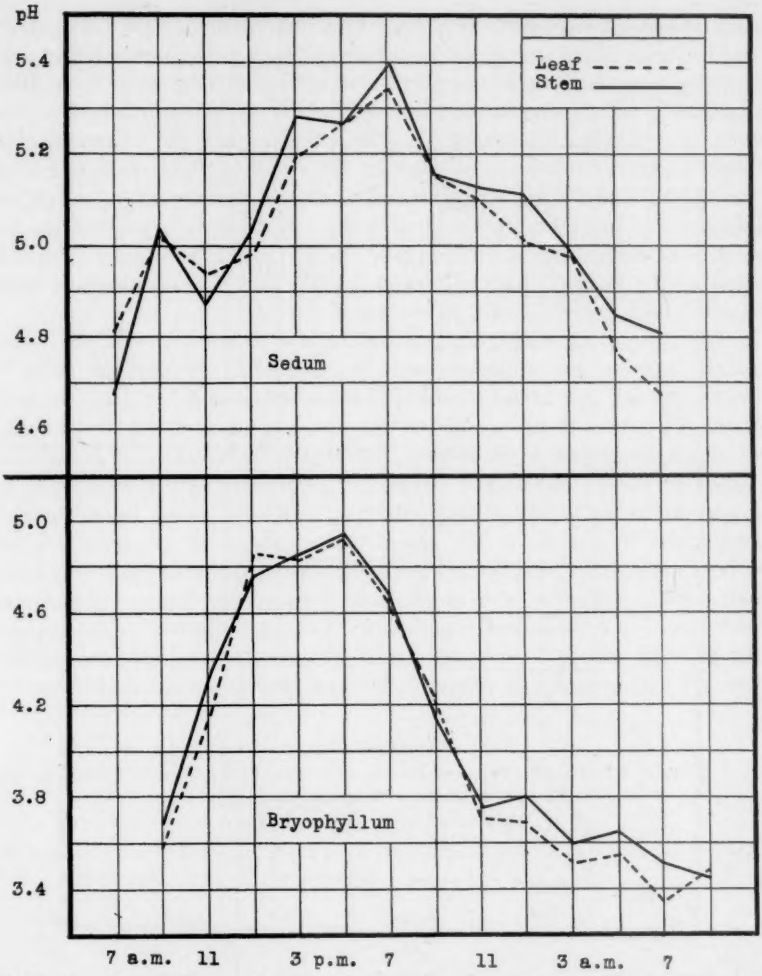
pH VALUES OF TISSUE FLUIDS OF STEMS AND LEAVES OF *Sedum*, *Bryophyllum* AND *Tradescantia* PLANTS AT TWO HOUR INTERVALS

TIME	*Sedum*		*Bryophyllum*		*Tradescantia*	
	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES
9:00 A. M.	5.131	5.122	3.685	3.584	4.641	4.395
11:00 A. M.	4.970	5.038	4.311	4.125	5.241	5.207
1:00 P. M.	5.122	5.081	4.750	4.852	5.528	5.453
3:00 P. M.	5.376	5.292	4.835	4.818	5.461	5.384
5:00 P. M.	5.359	5.359	4.936	4.920	5.511	5.495
7:00 P. M.	5.494	5.440	4.666	4.649	5.545	5.494
9:00 P. M.	5.249	5.249	4.159	4.243	5.148	5.173
11:00 P. M.	5.224	5.198	3.753	3.719	5.351	5.300
1:00 A. M.	5.207	5.114	3.821	3.685	5.275	5.266
3:00 A. M.	5.087	5.080	3.618	3.516	4.666	4.556
5:00 A. M.	4.869	4.945	3.652	3.550	4.548	4.717
7:00 A. M.	4.784	4.911	3.516	3.347	4.599	4.646
9:00 A. M.	.....	.....	3.449	3.483	4.954	4.903

from 4.91 at 7:00 A. M. to 5.46 at 5:00 P. M. In this plant the pH values of stem and leaf juices show no significant differences and the graphs representing these values follow somewhat the same course throughout. This appears to be characteristic of fleshy, thick leaved plants such as were here used.

Bryophyllum, another of the thick leaved, fleshy plants, shows an extreme range in the pH values of its tissue fluids from day to night, as is indicated by the graphs of figure 4. This range is more than double that indicated for *Sedum*. The maximum pH 4.92 for leaf juices occurred at 5:00 P. M. and the minimum pH 3.34 at 7:00 A. M., while the corresponding maximum and minimum values for stems, pH 4.93 and pH 3.44 are shown for 5:00 P. M. and 7:00 A. M., respectively. The graphs representing the courses of pH values for stems and leaves throughout the experimental period run quite closely together, but again there are no significant differences between stem and leaf values such as are indicated for the thin-leaved plants.

In table I and table II are presented also data for *Rumex* and *Tradescantia*, dealing with the influence of light intensity on the H-ion concentration of plant tissue fluid. These data are not here discussed, and are presented merely to emphasize the fact that acidity change with variation in light intensity is a phenomenon common to many types of plants and occurs



FIGS. 3 (upper) and 4 (lower). Graphs representing the course of change in pH values of leaf and stem juices of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

in proportion to the degree of succulency. This is further emphasized by tests of many species, the data for which are not here presented.

It has long been known that the succulent plants exhibit periodic rise and fall of the acid content of their juices, and the relation of this phenomenon to the respiratory processes has been the subject of extensive and thor-

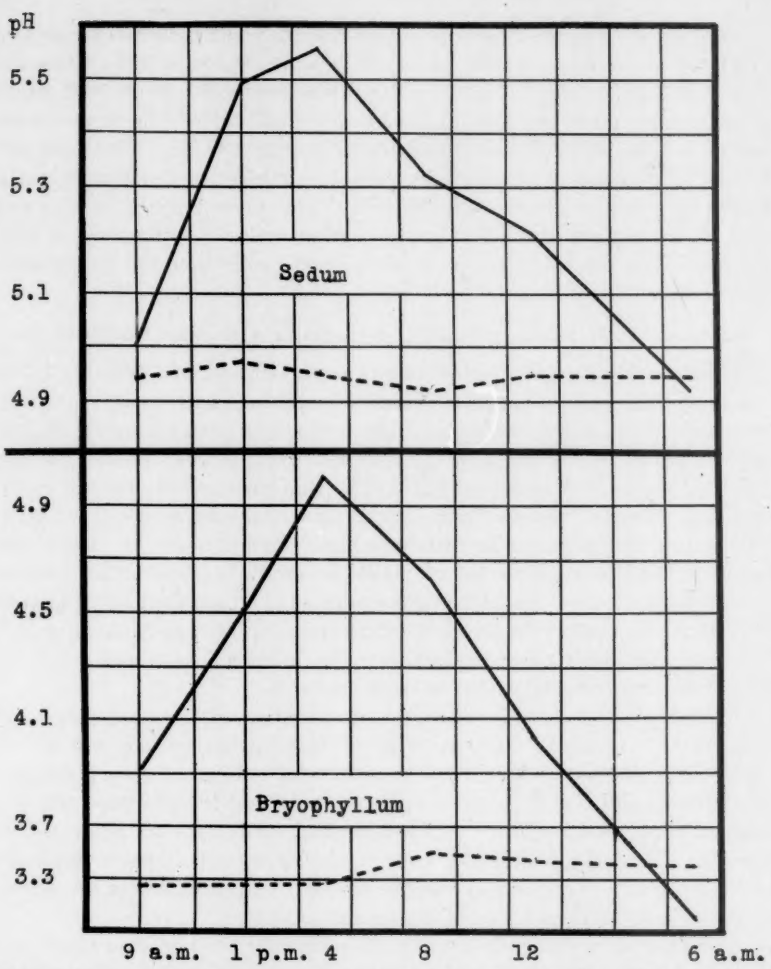
ough investigation. Both RICHARDS (19) and SPOEHR (23) have shown that the most important single factor which leads to the formation of acids in fleshy succulents is a low or insufficient oxygen supply, while their disappearance is mainly due to the photolytic action of light which breaks them down into simpler substances. But whether the low pH values of plant tissue fluids in the absence of light are the result of the formation and accumulation, under these conditions, of titratable organic acids, or whether they are due to the formation of carbonic acid from the accumulation and solution of carbon dioxide in the plant juices at night, as appears to be indicated by the work of MAQUENNE and DEMOUSSY (14) are questions which have not been experimentally investigated.

The present investigation, however, is not concerned with the causes underlying the accumulation and disappearance of organic acids in plants, but only with the fact that pH fluctuations of plant tissue fluids do occur, not only in fleshy succulents but also, to a lesser extent, in plants exhibiting a low degree of succulency, and that these fluctuations are directly related to variations in light intensity. To demonstrate that light is the important factor in the diurnal pH fluctuations of plant tissue fluids, a comparison was made of the juices of the plants of *Bryophyllum* and *Sedum*, exposed in the greenhouse to alternating day and night during 24 hour periods, with the juices of plants of the same age grown under similar conditions, but kept in dark chambers in the greenhouse during corresponding 24 hour periods. Care was taken to keep the temperatures approximately equal around the plants inside and outside of the dark chambers

TABLE III

pH VALUES OF THE TISSUE FLUIDS OF *Sedum* AND *Bryophyllum* PLANTS IN CONTINUOUS DARKNESS AS COMPARED WITH THOSE EXPOSED TO INTERMITTENT PERIODS OF LIGHT AND DARKNESS

TIME	*Sedum*		*Bryophyllum*	
	LIGHT	DARK	LIGHT	DARK
	pH	*pH*	*pH*	*pH*
9:00 A. M.	5.004	4.936	3.922	3.483
1:00 P. M.	5.495	4.970	4.514	3.483
4:00 P. M.	5.562	4.953	5.004	3.483
8:00 P. M.	5.325	4.920	4.632	3.615
12:00 P. M.	5.207	4.953	4.023	3.584
6:00 A. M.	4.920	4.936	3.347	3.550



FIGS. 5 (upper) and 6 (lower). Graphs representing the pH values of tissue fluids of *Sedum* and *Bryophyllum* plants which were exposed to intermittent (unbroken line) and continuous (broken line) periods of darkness.

during the experimental periods. Data for such a comparison are given in table III and are shown graphically in figures 5 and 6.

It will be observed that the juices of the plants exposed to alternate light and dark show the usual wide range in pH values, while the juices of the plants kept in continuous darkness show only very slight fluctuations which are not at all related to the light factor.

It may be of interest here to emphasize the point that comparisons of plant tissues or tissue fluids, particularly with respect to pH values and also, as will be brought out later, with respect to soluble iron content, can be of little value unless the samples upon which quantitative measurements are made are collected at the same time during the day or night. External conditions, particularly light intensity, which is subject to almost continuous fluctuation, have a pronounced influence upon these internal factors and may render any set of measurements of them useless for purposes of comparison unless careful attention is given to the collection and preparation of experimental material.

Relation of pH values to soluble iron content of plant tissue fluids

It has been suggested by HOFFER and CARR (**9**) that the mobility of iron and aluminum salts in plants is associated with high sap acidity, and they have shown that under certain conditions relatively large quantities of iron and aluminum will accumulate in different parts of corn plants. It has also been shown by MARSH and SHIVE (**15**) that plants under certain conditions may become chlorotic from lack of iron in the leaves when the total iron content of the plants is excessively high. Furthermore, in view of the fact that the iron requirement of plants is relatively high during periods of high light intensity and low during periods of diminished light, it is of interest to determine whether or not the soluble or *filterable* iron in plants bears any relation to the periodic fluctuation in the pH values of the tissue fluids resulting from variations in light intensity.

Accordingly, the soluble (filterable) iron content of tissue extracts taken at regular intervals throughout 24-hour experimental periods was determined for a number of species. The manner of taking samples, preparing the extracts, and the technique employed in making the pH tests and the chemical analyses have already been described. The data are presented in table IV. This experiment, like those previously described, was carried out on clear days so that the plants were exposed to approximately maximum variation in light intensity from day to night.

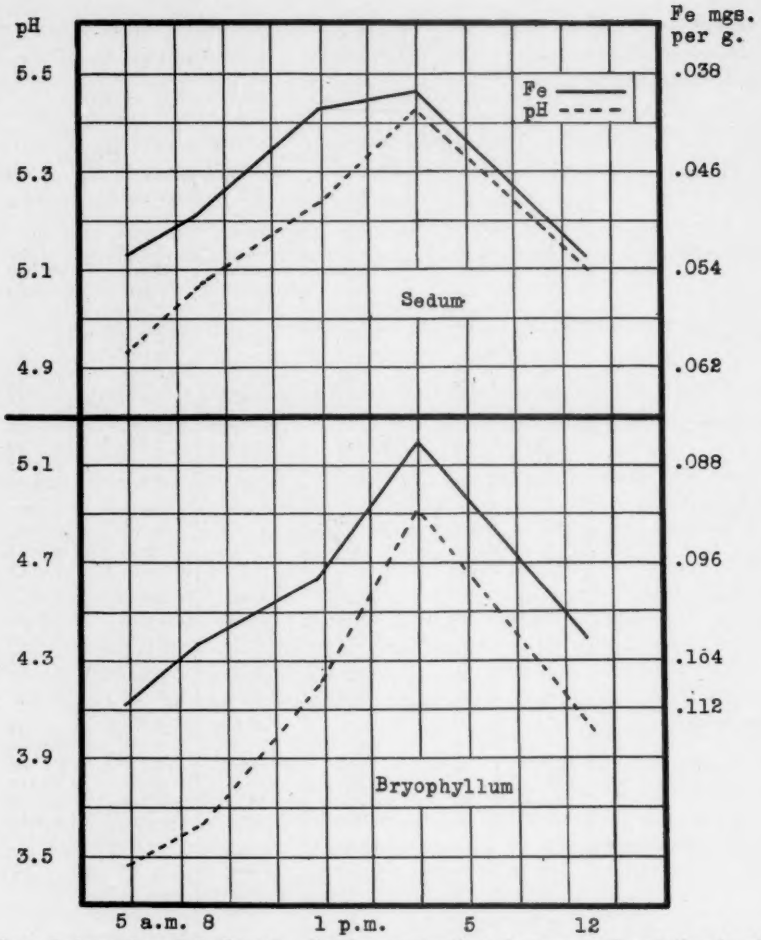
Examination of the data of table IV brings out the fact that there is a direct and very exact relation between the H-ion concentration of the tissue fluids and the soluble iron content of all the species investigated. In each species, the fluctuation in pH values of the plant juices with variation in light intensity is followed, in the inverse order, by a corresponding fluctuation in the soluble iron content. That is, for each species, low pH values correspond with high soluble iron content and high pH values with low soluble iron content.

To bring out the exactness of this relation and to show the course of fluctuation of the soluble iron content in the plants during a 24-hour period,

TABLE IV

pH VALUES AND SOLUBLE (FILTERABLE) IRON CONTENT, EXPRESSED AS MG. PER GRAM OF DRY TISSUE, AT INTERVALS DURING 24-HOUR EXPERIMENTAL PERIODS

Plant	Time	Stems		Leaves	
		Acidity	Fe	Acidity	Fe
		pH	*mg.*	*pH*	*mg.*
Asparagus	6:00 A. M.	5.478	0.0342	5.687	0.0299
	12:00 Noon	5.528	0.0277	5.850	0.0239
	5:00 P. M.	5.664	0.0245	6.086	0.0223
	12:00 Night	5.511	0.0302	5.816	0.0267
Clover	6:00 A. M.	5.630	0.0327	5.934	0.0316
	12:00 Noon	5.866	0.0202	6.137	0.0275
	5:00 P. M.	5.968	0.0241	6.357	0.0247
	12:00 Night	5.731	0.0329	5.985	0.0289
Soybean	6:00 A. M.	5.731	0.0354	5.883	0.0330
	12:00 Noon	5.782	0.0300	5.923	0.0299
	5:00 P. M.	5.830	0.0247	6.230	0.0212
	12:00 Night	5.799	0.0333	5.960	0.0244
Tobacco	6:00 A. M.			5.579	0.0438
	12:00 Noon			5.765	0.0315
	5:00 P. M.			5.833	0.0364
	12:00 Night			5.664	0.0445
Tomato	5:00 A. M.	5.190	0.0392	5.579	0.0314
	9:00 A. M.	5.207	0.0373	5.718	0.0299
	1:00 A. M.	5.461	0.0331	5.985	0.0256
	5:00 P. M.	5.562	0.0298	6.154	0.0237
	9:00 P. M.	5.528	0.0361	6.036	0.0266
	1:00 A. M.	5.308	0.0345	5.799	0.0295
Buckwheat	5:00 A. M.	4.395	0.0751	4.903	0.0588
	9:00 A. M.	4.427	0.0762	4.970	0.0566
	1:00 P. M.	4.598	0.0668	5.038	0.0489
	5:00 P. M.	4.804	0.0626	5.359	0.0445
	9:00 P. M.	4.767	0.0676	5.258	0.0518
	1:00 A. M.	4.362	0.0768	5.089	0.0588
Sedum, whole plant	5:00 A. M.	4.936	0.0535		
	8:00 A. M.	5.055	0.0502		
	1:00 P. M.	5.241	0.0429		
	5:00 P. M.	5.427	0.0399		
	12:00 Night	5.122	0.0535		
Bryophyllum	5:00 A. M.			3.483	0.1071
	8:00 A. M.			3.612	.....
	1:00 P. M.			4.210	0.0972
	5:00 P. M.			4.896	0.0861
	12:00 Night			4.023	0.1020
Dock, whole plant	5:00 A. M.	4.091	0.0762		
	8:00 A. M.	4.193	0.0640		
	1:00 P. M.	4.226	0.0557		
	5:00 P. M.	4.480	0.0513		
	12:00 Night	4.362	0.0640		



FIGS. 7 (upper) and 8 (lower). Graphs representing the course of variation in pH values and soluble iron content of *Sedum* and *Bryophyllum* plants during a 24-hour experimental interval.

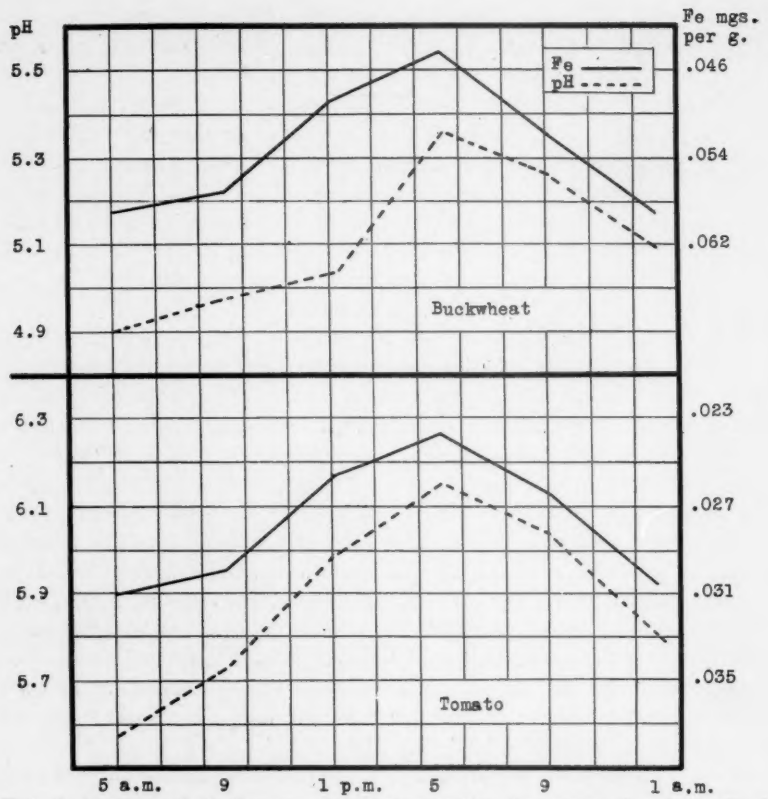
the data for two species of fleshy succulents (*Bryophyllum* and *Sedum*) and for two species of thin-leaved plants showing a relatively low degree of succulency (tomato and buckwheat) have been plotted to form the graphs of figures 7 and 8, and 9 and 10. The pH values and the values for the soluble iron content for each of these species are plotted together to form a pair of graphs with common abscissas, the ordinates on the left indicating

pH values, those on the right expressing soluble iron content (mg. per gram of dry plant tissue). To avoid intersecting of the graphs, the ordinates on the right are written in the inverted order.

The lower graph for each species (figs. 7 and 8) shows the usual course of pH change during a 24-hour period and this, in every case, is almost duplicated by the inverted graph representing the course of fluctuation in the soluble iron content during the same period, thus indicating an intimate relation between pH values of tissue fluids and that portion of iron in the plant which may be regarded as the active fraction, on the reasonable assumption that filterable iron here considered is mobile, readily available, and capable of functioning in the plant processes.

Another significant and important relation is here indicated. A comparison of the data for *Bryophyllum* with those for *Sedum* (fleshy succulents, figs. 7 and 8) brings out the fact that the juices of the former show relatively low pH values, ranging between 3.48 and 4.90, with a relatively very high content of filterable iron, ranging between 0.0861 and 0.1071 mg. per gram of dry tissue; while the juices of the latter show higher pH values, 4.94 to 5.43, with a correspondingly much lower content of filterable iron, ranging from 0.0399 to 0.0536 mg. per gram of dry tissue during a 24-hour period. A comparison of the data for tomato and buckwheat (thin-leaved plants with relatively low degree of succulency, figures 9 and 10) shows this relation in an equally definite manner. Of the four species graphically considered, the tomato shows the highest pH values, varying between 5.58 and 6.15, and the lowest content of filterable iron, fluctuating between 0.0314 and 0.0237 mg. per gram of dry tissue, during an experimental period of 24 hours. This relation holds for all the species the data for which are presented in table IV. Furthermore, the relation holds also for different organs of the same plant, as between stems and leaves, when these organs show considerable difference in the pH values of their juices. This is clearly shown by the data in table IV for stems and leaves of buckwheat, tomato, asparagus and soybeans. The significance of this relation will be further considered in the following section.

It might be well here to suggest that from the data thus far presented it appears that those plants in which the pH values of the tissue fluids lie close to the precipitation point of iron (about 6.0) show greater fluctuation in the filterable iron content from day to night than do those plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron. This is probably to be inferred from the fact, as will be brought out later, that in plants of the latter type a high percentage of the total iron is in the soluble form, and under such conditions small fluctuations in this fraction might be expected. However, this is merely put forth as



FIGS. 9 (upper) and 10 (lower). Graphs representing the course of variation in pH values and soluble iron content of buckwheat and tomato leaves during a 24-hour experimental interval.

a suggestion, since not sufficient evidence of a positive character is at hand to warrant a definite conclusion.

Comparison of soluble and total iron content of plants with pH of tissue fluids

It has already been pointed out that in certain types of plants, only a very low percentage of the total iron in the tissues is in the soluble (filterable) state, while in other types of plants nearly all the iron is in the soluble state. A study was made of a number of species showing a range in the average pH values of tissue fluids from 4.04 to 6.10, in order to emphasize the relation which pH values of the tissue fluids bear to total, insoluble, and soluble iron fractions in the various species.

TABLE V

AVERAGE pH VALUES, TOTAL AND SOLUBLE IRON CONTENT OF VARIOUS PLANTS OVER A 24-HOUR PERIOD

PLANT	LEAF			STEM		
		Fe, PER GRAM OF DRY TISSUE			Fe, PER GRAM OF DRY TISSUE	
	ACIDITY	TOTAL	SOLUBLE	ACIDITY	TOTAL	SOLUBLE
	pH	*mg.*	*mg.*	*pH*	*mg.*	*mg.*
Bryophyllum	4.04	0.137	0.0958			
Rumex (whole plant)	4.24	0.219	0.0622			
Buckwheat	5.10	0.269	0.0516	4.58	0.082	0.0696
Sedum (whole plant)	5.16	0.278	0.0477			
Tobacco	5.74	0.325	0.0390			
Tomato	5.87	0.297	0.0277	5.38	0.089	0.0349
Asparagus	5.87	0.373	0.0256	5.54	0.111	0.0214
Soybeans	6.01	0.469	0.0246	5.79	0.142	0.0308
Clover	6.10	0.571	0.0281	5.78	0.214	0.0299

The data for the various species presented in table V are arranged in the ascending order of average pH values of the different species. The data were obtained by collecting samples of each species at regular intervals (two or four hour intervals) throughout twenty-four hour periods on clear days. The various measurements were made on these samples and the corresponding values obtained for each species were then averaged; so that each value in the table represents the average of the values obtained over a twenty-four hour period.

It will be observed that the total iron content increases in the different species as the pH value of the tissue fluids increases: that is, high total iron in any given species corresponds to high pH value of the tissue fluids and low total iron with low pH values. Thus, *Bryophyllum* with the low average pH value of 4.04, shows also a relatively low total iron content of 0.137 mg. per gram of dry tissue; while clover with a pH value of 6.10, shows the abnormally high total iron content of 0.571 mg. per gram of dry tissue. On the other hand, the soluble iron content of the different species varies in the inverse order with variation in pH values of the tissue fluids. That is, low pH values correspond to high soluble iron, and high pH values correspond with low soluble iron. Thus, *Bryophyllum* with a low average pH value of 4.04 shows a low total iron content (0.137) but relatively high soluble iron (0.0958); while clover with a pH of 6.10 shows the abnormally high total iron of 0.571 mg. per gram of dry tissue but a very low content of soluble iron (0.0281). These relations hold, not only for the different species, but

also for different organs of the same plant, as between stems and leaves when these organs show considerable difference in the pH values of their tissue fluids, as has already been pointed out.

From the foregoing considerations it is quite apparent that plants like *Bryophyllum*, *Rumex* and others with relatively low pH values of the tissue fluids, absorb only very small quantities of iron, and that practically all of the iron absorbed remains in a soluble form and is presumably available and capable of functioning in chlorophyll production and other plant processes. But plants such as clover, soybeans, and others with high pH values of the tissue fluids absorb relatively very large quantities of iron, if this is available in the external medium, but only a small proportion of this remains in a soluble form in the plant. Much of the total iron in plants like these is precipitated, probably along paths of translocation, and is therefore unavailable and undoubtedly does not function in the plant processes. If, for any reason, all of the iron in plants of this type should become soluble at any one time, iron toxicity would probably follow and might result in the death of the plant.

The cause for the accumulation of relatively large quantities of unavailable iron in the tissues of plants in which the pH values of the sap lie close to the precipitation point of iron, or the mechanism by which this is accomplished, is at present not clear. It may be suggested, however, that through the precipitation of iron in the plant tissues this element is removed from the field of osmotic activity and thereby a diffusion gradient for it may be maintained from the outside to the inside of the plant, resulting in the accumulation of relatively large quantities of non-available iron.

From the data here presented, it is to be expected that plants in which the pH values of the tissue fluids lie close to or above the precipitation point of iron may yield a high total iron content, but only a small proportion of this total iron can function in chlorophyll production and other plant activities. This is made clear, not only by chemical analyses of the tissues and tissue fluids, but also by the fact that chlorosis is likely to occur in these plants from lack of available iron under slightly unfavorable conditions, and particularly under conditions of high light intensity during periods of which the plant sap attains its maximum pH value and the plant its minimum value for soluble iron. On the other hand, plants in which the pH values of the tissue fluids lie considerably below the precipitation point of iron show relatively low total iron, but nearly all this iron can be extracted with the tissue fluid and is capable of passing through a quantitative filter-paper of the highest quality. This iron appears to be quite mobile in the plant and is uniformly distributed, as is indicated by quantitative analyses of the tissues of the various plant organs, and it is therefore rea-

sonable to assume that this iron is capable of functioning in the plant processes.

Summary

1. The hydrogen ion concentration of tissue fluids varies with light intensity: low hydrogen ion concentration corresponds to high light intensity, and high hydrogen ion concentration to low light intensity.

2. Fleshy or succulent plants show greater variation in hydrogen ion concentration of tissue fluids with change in light intensity than do thin leaved plants, the range of variation occurring in proportion to the degree of succulency of the plants.

3. All plants studied show differences in hydrogen ion concentration between leaf juices and stem juices; fleshy or succulent plants show much lower differences, however, than do non-succulent plants, the degree of difference being in proportion to the degree of succulency.

4. A comparison of hydrogen ion concentration of tissue fluids of different species has no significance whatever unless determinations are made from material collected at the same time from plants grown under approximately identical conditions.

5. Soluble (filterable) iron content of plants varies directly with the hydrogen ion concentration variation brought about by changes in light intensity from day to night.

6. Plants in which the tissue fluids have low hydrogen ion concentration values show high total and relatively low soluble iron content; and those in which the tissue fluids have high hydrogen ion concentration values show low total iron and relatively high soluble iron content.

7. In all plants studied, the iron content of leaves is higher than the iron content of stems.

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PHENOLASE ACTIVITY IN RELATION TO SEED VIABILITY

W. C. DAVIS

(WITH THREE FIGURES)

Introduction

Oxidase and catalase activity of plant tissues are so frequently used as criteria of metabolism and viability that methods for their quantitative measurement are becoming increasingly important in physiological technique. Quantitative data for catalase, however, are more numerous than those of other oxidases, probably because of the difficulty encountered in making accurate determinations of oxygenases and peroxidases. There are, nevertheless, several important reasons for more frequent estimations of oxidase activity. The physiological function of catalase is still obscure. Oxidases, on the other hand, have been closely associated with the ability of living organisms to bring about transformations of materials otherwise stable at ordinary temperatures. Though our knowledge of oxidation-reduction reactions *in vivo* is as yet fragmentary, the abundant and diverse experimental evidence which has accumulated makes inescapable the conclusion that oxidases are essential to co-ordinated respiratory and other oxidative activities of plants. The correlation between oxidases and physiological processes has been so definite that these enzymes have been placed in a causal relationship to them. The desirability of a convenient quantitative method for oxidase determination is consequently self-evident.

Colorimetric methods for estimation of oxidases have been widely used. These methods have involved the use of such reagents as alpha-naphthol, hydroquinon, pyrogallol, paracresol, ortho- and para-aminophenol and others. Though solutions of these reagents are to varying degrees autoxidizable in air, the amount of spontaneous oxidation can be minimized by keeping the hydrion concentration within definite limits for each reagent (6). All of them, however, are appreciably autoxidized in nearly neutral media. This is especially true of two common reagents, phenylenediamine hydrochloride and demethyl-phenylenediamine, which have as a consequence been little used as such in colorimetric estimations of oxidases.

In an attempt to procure an understanding of enzymatic processes in plants, objection arises to any appreciable modification of the hydrion concentration of the reaction medium from that approximately normal to the plant. Realization of this fact has resulted in a search for reagents which are not greatly autoxidized in neutral media (**6**). A few oxidase reagents of this type, such as alpha-naphthol and paracresol are available but they do not produce the steep color gradients of more sensitive chromogens. Other

things being equal, sensitive reagents reveal more minute differences and produce sharper contrasts. These characteristics and the fact that the procedure with certain precautions can be made quantitative readily justify the use of these reagents despite the apparent complexity of the process. An experiment was undertaken with the Nadi reagent to determine its utility in the estimation of indophenol oxidase activity in certain plant tissues. This reagent has found general application in animal physiology but only infrequent use thus far in plant problems (**7, 9, 10, 12**). This report describes the use of the Nadi reagent in a study of seed viability.

Experimental procedure

The colorimetric procedure for determination of phenolase activity involved essentially the following steps, namely, the preparation of a completely oxidized color standard in terms of which the phenolase activity and spontaneous autoxidation are expressed, the determination of the phenolase activity of a unit quantity of seed powder, and the spontaneous autoxidation of the reagent containing a unit quantity of inactivated seed powder. The colorimetric reading of the latter was then subtracted from the reading obtained for the active enzymatic material and the result recorded as the actual phenolase activity.

The color standard was prepared by taking 125 cc. of M/100 α-phenylenediamine, an equal amount of M/100 α-naphthol, 62 cc. of 0.25 per cent. sodium carbonate and placing them in a 500-cc. bottle through which washed air was vigorously bubbled for five days to insure maximum coloration. The solution was then made up by volume to 70 per cent. alcohol. It was observed that the percentage of alcohol, acidity, and alkalinity of the solution greatly influenced the color. The resulting oxidized solution had a pH of 7.0. Since the accuracy of the colorimeter, other things remaining constant, depends upon having nearly the same color intensity in the standard and unknown, the two were always balanced as nearly as possible. Dilutions of the standard made were primarily for the purpose of increasing the accuracy of the colorimeter determinations.

A series of convenient dilutions was made by pipetting 1 cc. of the completely oxidized Nadi reagent into 40 cc. of 70 per cent. alcohol, 1 in 60, 1 in 80, 1 in 100, 1 in 150, 1 in 500, and 1 in 1000. These color standards were designated in the order given as 40, 60, 80, etc. The enzyme powder was in all cases maintained at a concentration of 1 per cent. while the intensity of the color standard employed was chosen with respect to the phenolase activity.

A small number of assorted seeds was ground in a Nixtamal mill, only seeds apparently free from mechanical injury, and infection being selected. Each group of seeds was labelled to identify it in relation to variety

and age. Lots of seeds were then analyzed at random to avoid possible error due to preconceived notions. The powder was then screened through an 80 mesh sieve. In the case of wheat a few determinations were made with coarser material which passed through the 40 mesh but not the 80 mesh screen. In all other determinations only the material which passed the 80 mesh screen was used, the object being to secure material as nearly uniform in physical texture and composition as possible. The powder, though no definite attempt was made to differentiate the various parts of the seeds, was predominantly endosperm and embryo tissue.

In determination of phenolase activity the sieved powder was weighed and made up to 1 per cent. by volume with boiled, distilled water at pH 6.8 (**2, 3, 14**). This solution was then allowed to stand for 90 minutes. In the meantime small quantities of dimethyl-para-phenylenediamine hydrochloride (hereafter referred to as phenylenediamine), α-naphthol, and sodium carbonate were weighed. The sodium carbonate was used to neutralize the acidity of the phenylenediamine. When the aqueous enzyme solution has stood for 90 minutes at room temperature (23° C. to 26° C.) the weighed quantities of α-naphthol and phenylenediamine were made up with distilled water to a concentration of M/100. This is approximately the concentration employed by other workers in similar investigatons (**5, 7, 10**), and it was found to give satisfactory results with the seeds used in this experiment.

The phenylenediamine and α-naphthol solutions were not made up and mixed until used as they are relatively unstable and autoxidizable in the presence of air. The substances, however, are quite stable in the solid form. α-naphthol as used in this experiment was made up by adding equal parts of distilled water and 95 per cent. alchohol. An aqueous solution of α-naphthol was also prepared by boiling 8 gm. of the solid α-naphthol in 1 liter of distilled water for about 150 minutes. The solution was then made up to 1 liter by volume, cooled, and filtered. A few determinations were then made to see if the 9 per cent. alcohol retarded the reaction. No retardation was observed and consequently the alcoholic solution of α-naphthol was used in all of the experimental work. It was found that 1 cc. of 0.25 per cent. sodium carbonate was sufficient to neutralize the phenylenediamine, giving the final pH as 7.5 for the total substrate. A 2-cc. portion of α-naphthol, an equal amount of phenylenediamine, 1 cc. of sodium carbonate and 5 cc. of an infusion made from 1 part of seed powder in 100 parts of distilled water were then pipetted into a 9.5 cm. petri dish. The shallow petri dish was used to increase the surface area in contact with air (**5**). The reaction was allowed to proceed for a given period of time with occasional shaking and the solution was then made up by volume to a 70 per cent. alcohol concen-

tration. The frequency and duration of shaking were constant for each group of seeds.

It was observed that the increase in the alcoholic concentration inhibited the enzyme action but did not stop the autoxidation of the reagents. A time period of ten minutes was adequate for the resulting di-amino-compound to become uniformly dispersed in the alcohol. DYE (5) working with animal tissues found it necessary to allow 15 minutes for complete dispersion of indophenol in alcohol. A difference in the physico-chemical properties in plant and animal extracts may account for the difference in the time period required for the reaction.

A volume of the colored solution was then decanted and taken out of the petri dish and its color intensity determined in a Duboscq colorimeter against the color standard. For a control, 2 cc. of phenylenediamine and equal volume of α-naphthol, 1 cc. of sodium carbonate, and 5 cc. of enzyme solution were pipetted into a petri dish and made up by volume to 70 per cent. alcohol. This made the concentration of the control similar to that of the unknown, thus affording a fairly accurate measure of the spontaneous oxidation of the Nadi reagents in the presence of atmospheric oxygen. Temperature variations in all of the experimental work remained in the range of 22.9 to 26.3° C. The per cent. oxidation of indophenol which might be formed was divided into the actual amount formed as determined in the colorimeter, and this was expressed as percentage.

In the determination of catalase activity an apparatus similar to that employed by APPLEMAN (1) was used. The determinations were made by placing 1 gm. of the seed powder in a 250-cc. Erlenmeyer flask, with 20 cc. of hydrogen peroxide, with reaction period (ten minutes) and the shaking constant throughout. Corrections for variations in atmospheric pressure were made and the corrected gas volume was recorded.

The per cent. germination of seeds was taken by selecting multiple lots of 25 apparently perfect seeds. These were then treated for 5 minutes in 0.5 per cent. mercuric chloride solution, washed with distilled water, and then placed in sterile petri dishes containing a small amount of uniformly moistened absorbent cotton. The seeds were at all times subjected to a saturated atmosphere. A germination count was made at the end of each day for 5 days. Temperature variation during the germination period was from 25 to 34° C. All seeds remained free from infection during the germination period.

The seeds used in the experiments had been stored under ordinary laboratory conditions and although no attempt had been made to provide absolutely uniform conditions throughout the period of storage, they were not exposed to abrupt temperature or moisture fluctuations.

The experimental data are presented in the accompanying tables I–V.

TABLE I

AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	POWDER	REACTION TEMPERATURE	OXIDATION	GERMINATION
		MARQUIS WHEAT		
years	*mesh*	*°C.*	*per cent.*	*per cent.*
3	80	23.8	14	24
5	80	23.1	23	60
6	80	23.0	24	76
7	80	23.0	16	28
8	80	23.1	13	12
3	40	23.5	9	24
5	40	23.0	25	60
6	40	22.9	24	76
7	40	23.4	14	28
8	40	23.3	13	12
		MICHIGAN AMBER WHEAT		
1	80	25.7	21.8	32
6	80	25.6	21.4	0
13	80	25.6	13.0	0
1	40	25.8	16.0	32
6	40	25.8	12.0	0
13	40	25.8	10.0	0

TABLE II

AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	REACTION TEMPERATURE	OXIDATION	GERMINATION
	PINK OF WINTER WHEAT		
years	*°C.*	*per cent.*	*per cent.*
1	25.1	50	64
3	25.0	29	8
4	24.3	39	32
6	24.4	18	0
1	25.1	37	64
3	25.0	33	8
4	24.2	29	32
6	24.8	26	0
	SUCCESS BEARDLESS BARLEY		
1	25.9	23	64
4	25.7	22	12
11	26.1	18	8

TABLE III

AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	REACTION TEMPERATURE	TIME		OXIDATION	GERMINATION
		REACTION	TOTAL		
SWEDISH SELECT SPRING OATS					
years	*°C.*	*min.*	*min.*	*per cent.*	*per cent.*
1	25.5	10	20	18.33	16
2	25.5	10	20	18.19	16
3	25.9	10	20	16.86	52
4	25.5	10	20	16.21	52
5	25.5	10	20	14.00	12
6	25.3	10	20	15.10	16
7	25.3	10	20	12.76	0
8	25.2	10	20	12.00	0
9	25.1	10	20	10.45	0
1	25.3	45	60	75.90	16
2	25.3	45	60	75.60	12
5	25.3	45	60	75.80	0
7	25.3	45	60	75.96	0

TABLE IV

AGE, PHENOLASE ACTIVITY, AND GERMINATION

AGE	TEMPERATURES		TIME		OXIDATION	GERMINATION
	INCUBATION	REACTION	REACTION	TOTAL		
ARLINGTON WHITE SPINE CUCUMBER						
years	*°C.*	*°C.*	*min.*	*min.*	*per cent.*	*per cent.*
2	25.4	25.4	20	40	27.0	56
3	25.4	25.4	20	40	26.8	52
4	25.4	25.4	20	40	27.2	28
2	26.7	26.1	10	20	15.0	56
3	26.1	26.1	10	20	14.0	52
4	26.1	26.1	10	20	10.0	28
2	52	26.3	10	20	13.0	56
3	52	26.3	10	20	12.0	52
4	52	26.3	10	20	8.0	28
MANCHURIA SPRING BARLEY						
1	25.9	25.9	10	20	34	76
4	25.9	25.9	10	20	27	46
11	25.9	25.9	10	20	24	12
1	52	26.4	10	20	30	76
4	52	26.4	10	20	26	46
11	52	26.4	10	20	24	12

TABLE V

RELATION OF CATALASE ACTIVITY AND AGE IN SEEDS

AGE	AMOUNT	POWDER	AMOUNT OF GAS	GERMINATION
		ARLINGTON WHITE SPINE CUCUMBER		
years	*gm.*	*mesh*	*cc.*	*per cent.*
4	0.2	40	14.55	56
4	0.4	40	26.4	56
3	0.2	40	14.8	52
3	0.4	40	32.0	52
2	0.2	40	15.1	28
2	0.4	40	33.1	28
		MICHIGAN AMBER WHEAT		
1	0.4	60	34.0	32
6	0.4	60	27.5	0
13	0.4	60	17.0	0

Discussion

In view of differences in interpretation of the indophenol reaction (7) and because of the exploratory character of the present investigation no attempt has been made in this report to adopt a critical definition of the terms oxidase and phenolase. The terms are employed simply to designate the oxidizing components of certain seeds. Although there is a certain amount of doubt regarding the exact course of the Nadi reaction there is consensus of opinion about the principle involved and the nature of the end products. It seems entirely justifiable, therefore, to employ the Nadi method in testing the phenolase activity of seeds. The Nadi method has already been proved experimentally satisfactory, it is quantitative, and there is agreement concerning the fundamental character of the reaction.

Analysis of the data (tables I–V) reveals that there is no simple quantitative correlation between phenolase activity, age and germination as determined by the Nadi reaction. If, however, both the age and percentage germination are taken into consideration then a definite though not proportional relationship becomes apparent. Those samples which were taken from relatively young seeds and had a high degree of germination gave correspondingly a large oxidation percentage. The fact revealed in table I, that the three year old seeds, in the case of Marquis wheat, have a lower percentage oxidation than 5 year old seeds is explained, in part at least, by the fact that the percentage of germination of the 5 year old grain is more than twice as great as that of the 3 year old. Oxidase activity consequently was in this instance more closely correlated with viability than with age.

Similar results have been reported by CROCKER and HARRINGTON (**4**) for *Amaranthus retroflexus*. Examination of the experimental results obtained from the 7 year old wheat with a germination of 28 per cent. as constrasted to 8 year old grain with 12 per cent. germination shows a decrease in oxidation as the concomitant of age and low vitality.

A difference in the amount of oxidation with variation in size of the mesh through which the seed powder has passed was also noted (table I). The powder which passed through the 40 but not the 80 mesh sieve gave more irregular results for both Marquis and Michigan amber wheat. While the percentage oxidation was more variable in the 40 mesh than in the 80 mesh powder it will be noted that the relationship to age and percentage germination of the seeds had the same general trend. The percentage of oxidation for the two varieties of wheat at the same age is not identical nor is there a definite correlation of Marquis and Michigan wheat in relation to germination and oxidation. It will also be noted (table I) that the 6 and 15 year old Michigan amber wheat did not germinate but that the latter gave a lower oxidation percentage. This indicates that there is a definite relation between age and phenolase activity.

The parallelism in catalase and phenolase activity in seeds of given age is of interest. The latter may prove to be the more reliable index to viability if the procedure for its determination can be standardized and simplified.

There appears to be a positive relationship between catalase and phenolase activity in Arlington white spine cucumber and Michigan amber wheat (fig. 1). The phenolase and catalase activity approximately parallel each other in relation to the age of seeds. The decrease in activity of the two enzymes accompanying senescence indicates that the intensity of the catalase

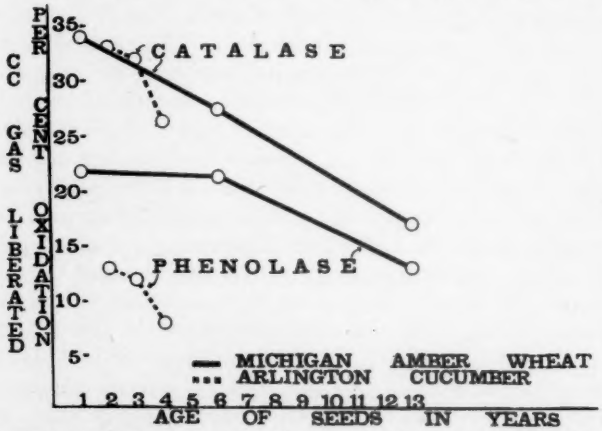

FIG. 1. Comparison of phenolase and catalase activity in seeds of wheat and cucumbers.

activity had no appreciable effect upon the amount of indophenol produced (**7**).

The same generalizations in relation to age and per cent. of germination apply to the Pink of Winter wheat (table II). The three year old Pink of Winter wheat with 8 per cent. germination gave a lower percentage oxidation than the four year old wheat of the same variety but with 32 per cent. germination. This situation is similar to that occurring in Marquis wheat (table I). These two cases indicate that there is a greater amount of correlation between oxidation and germination than between age and oxidation in the case of wheat. Success beardless barley, on the other hand (table II), disclosed a slow decrease in the rate of oxidation in relation to both age and germination for the first 4 years and then a relatively large decrease in relation to viability and senescence.

The drop in percentage oxidation is shown graphically (fig. 2). Barley, oats, and cucumber show a definite decrease in the phenolase activity in relation to age of the seeds. In wheat, on the other hand, there is an initial increase in the percentage oxidation which is subsequently followed by a decrease. It is, however, to be noted that the increase in the phenolase activity of wheat is also accompanied by an increase in the percentage germination. In the other determinations (fig. 2) the germination percentage de-

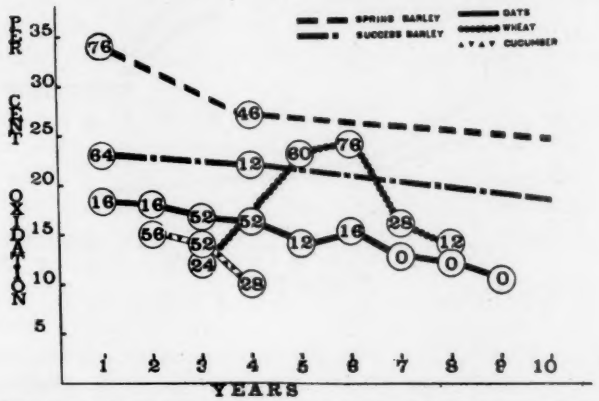

FIG. 2. Phenolase activity curves for various seeds of different ages. The numbers in the circles give the per cent. germination.

creases as the age increases in all species except oats. In this case, despite the increase in the per cent. germination, there is a decrease in degree of oxidation with age.

HARRISON (**7**) and other workers (**8, 11**) have shown that the indophenol formed in the Nadi reaction may in turn react with reducing substances

such as glutathione and hypoxanthine, being thereby reduced to its colorless leuco base. The final quantity of indophenol thus may be no reliable criterion of peroxidase activity if the concentration of reducing substances present is high. In a study of the type here reported, the utility of the Nadi reaction is not lost on this account, because reduction reactions in dormant seeds are inappreciable in comparison with those in actively growing tissues. The dilutions of the seed extracts and the brief time allowed for the reaction still further minimized reduction of indophenol.

Error of greater magnitude may possibly be introduced into the Nadi reaction by the presence of an appreciable amount of catalase, due to its decomposition of the peroxide upon which the formation of indophenol depends. Several investigations (**7, 10**) have shown that the inactivation of the more thermosensitive catalase at moderate temperatures actually results in an increase of indophenol by peroxidases. To test the effect of catalase on the formation of indophenol by phenolase, several samples of enzymatic seed extracts were subjected to a mild heat treatment.

Heat treatment at 52° C. for 90 minutes in a water bath without shaking (**7, 10**) decreased rather than increased phenolase activity in Manchuria spring barley and Arlington white spine cucumber (fig. 3). In the case of

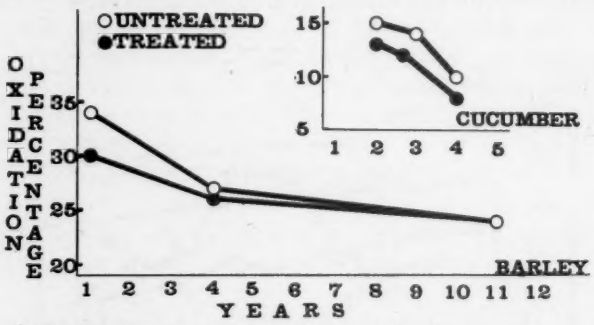

FIG. 3. Effect of heat treatment on phenolase activity.

barley of considerable age, the temperature treatment had very little effect upon the rate of oxidation. The effects of catalase in the dilutions of seed extracts employed thus appear to have been negligible.

The results of catalase determinations (table V) show a decrease in activity with age and low percentages of germination. Many experiments have been made on catalase in search of a method to determine viability, especially of seeds. SAMPIETRO (**13**) and CROCKER and HARRINGTON (4) show a relationship between catalase activity, age and germination. The correlation, however, is not definite enough to make reliable forecasts of the

germination percentage. The greater part of the work so far done with catalase in relation to germination apparently has been unable to evaluate precisely the effect of growth and storage conditions on enzymatic activity.

In conclusion, the Nadi reaction, involving short reaction periods and dilutions of seed extracts as above described, was found to yield concordant results in the study of phenolase activity of dry seeds of varying age. When the limitations of the Nadi reaction are circumvented, its sensivity makes it a simple and valuable indicator of oxidase activity. Phenolase activity as herein determined showed a high degree of correlation with seed viability.

Summary

A modification of the Nadi reaction for phenolase determination is described. Phenolase activity was high in young seeds with a high percentage of germination and low in old seeds of low percentage of germination. Enzymatic material heat treated at 25° C. for 90 minutes did not show increase in phenolase activity. Phenolase and catalase activity in general were parallel in cucumber and wheat.

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USE OF EXPRESSED SAP IN PHYSIOLOGIC STUDIES OF CORN[1]

J. D. Sayre and V. H. Morris

(WITH FOUR FIGURES)

In planning an extensive series of physiologic studies with corn, the need was felt for rapid, simple, yet accurate methods of making certain determinations. Plant physiologists have been using sap expressed under high pressures in various physiologic studies for many years. The apparatus and methods used and the results obtained have differed so widely that it was considered desirable to try different methods to find one that could be used satisfactorily in the investigations in mind. A description of the apparatus and procedure found satisfactory in obtaining samples of sap from vegetative parts of the corn plant suitable for some phases of the investigations, and data showing some of the limits within which such samples may be used are presented in this paper.

Apparatus and methods

A small laboratory hydraulic press (fig. 1) was used for expressing the sap from the corn tissues. A cage to contain the tissues, similar to that described by Meyer (**2**), was constructed so that the 25,000 pounds total working load exerted a pressure of 5,000 pounds per square inch on the tissue in the cage. The apparatus was arranged so that the sap obtained was filtered. The filter consisted of a layer of linen cloth and a layer of 100-mesh copper sieve wire placed over a perforated steel disk in the bottom of the cage. The disk was perforated with one-sixteenth inch holes, arranged radially in rows, with grooves on the lower side of the disk for drainage. Filtered through this, the sap contained no cell residue and was practically free from suspended material. The cage was sealed liquid tight by a rubber stopper placed above the tissue.

A uniform time of draining was found necessary for obtaining comparable results. If many samples are to be examined this time must be a minimum. In these experiments five minutes were allowed for draining, during which time the pressure was maintained at 25,000 pounds; this was long enough to express practically all of the obtainable sap.

In some of the experiments it was desired to test successive portions of the sap. The successive 10-cc. samples of sap from 100 grams of tissue were collected in separate 10-cc. graduated cylinders. The same process

[1] Based on investigations cooperative between the Ohio State Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

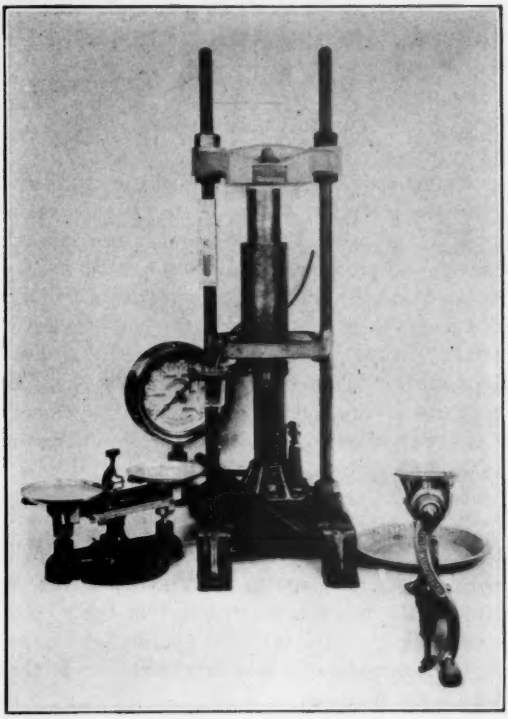

FIG. 1. Equipment used for obtaining samples of expressed sap from corn tissue.

was repeated with two other 100-gram samples of tissue and the corresponding portions from the three expressions were mixed to provide samples for examination.

Total solids in the sap were determined with the refractometer, GORTNER and HOFFMAN (1). The total reducing power of the sap was determined by a modification of the QUISUMBING-THOMAS method (3) after inversion of the sucrose by invertase. In analyzing the plant tissue for sugars for comparison with estimates based on the sap analyses the samples, which had been preserved in alcohol, were extracted with 80 per cent. alcohol and the total reducing power determined as above. In the comparisons shown this is designated as the "standard" method in contrast to the "sap" method.

Results

The experiments fall naturally into studies of (1) factors involving the amount of sap or water expressed, (2) the sugars and total solids in the sap

and in successive portions thereof, and (3) the relation between the sugars in the sap and in the tissue. The results with sucrose and with free reducing sugars were so alike that they have been combined, and only total sugars are reported.

The amount of sap expressed

Among the factors affecting the amount of sap which could be expressed studies were made of the preliminary treatment of the tissue, the size of sample, and the kind of tissue and its moisture content.

Effects of preliminary treatment.—Various methods of treating the tissue prior to expressing the sap have been used by other workers. These methods have been summarized by Meyer (**2**). Here, preparation by grinding the tissue with a food grinder (fig. 1) and mincing it, with and without freezing it to −9° C. in each case were compared. The grams of sap recovered from 100 grams of blade tissue of Burr-Leaming corn and of orchard grass after the different treatments are shown in table I.

TABLE I

The amount of sap expressed from blade tissue of corn and of orchard grass following different treatments
(Average of three determinations)

Blade tissue from	Amount of sap from 100 grams of tissue			
	Ground	Ground and frozen	Minced	Minced and frozen
	gm.	*gm.*	*gm.*	*gm.*
Corn	71	72	26	45
Orchard grass	53	58	13	33

More sap was recovered after grinding only than after mincing, either with or without freezing. Moreover, the additional amount of sap recovered after grinding and freezing the tissue, above that obtained after grinding only, was negligible. Grinding is more simple and rapid, and omission of freezing avoids any danger of change in the colloids that might result from that process. Finally, as will be shown later, successive portions of sap were more nearly alike after grinding than after mincing. Grinding seemed to be the preferable method of preparation and accordingly was used in the succeeding experiments reported in this paper.

THE INFLUENCE OF SIZE OF SAMPLE.—Using uniform pressures and times of draining, samples of 50, 100, and 150 grams were compared. Representative data for different tissues are given in table II.

TABLE II

PERCENTAGE OF TOTAL WEIGHT EXPRESSED AS SAP FROM DIFFERENT SIZED SAMPLES OF DIFFERENT CORN TISSUES

KIND OF TISSUE	PERCENTAGE TOTAL WEIGHT EXPRESSED FROM SAMPLES OF:		
	50 GRAMS	100 GRAMS	150 GRAMS
	per cent.	*per cent.*	*per cent.*
Stem	82	80	80
Blade	78	72	66

Larger percentages of sap were removed from the smaller samples, particularly of the blade tissue. As will be shown later, the sap remaining in the press cake is similar in composition, in certain respects, to that expressed. Accordingly, the 100-gram sample was used in our experiments in order to provide adequate quantities of sap and moderately high expression. For some purposes it probably would be better to obtain the necessary sap from two smaller samples.

KIND OF TISSUE AND MOISTURE CONTENT.—The amount of sap removed by pressure from blade and stem tissue of Burr-Leaming corn at different stages in its development is shown graphically in figure 2. The amount of sap obtained has been expressed as cubic centimeters of water (by subtracting the total solids from the weight of sap obtained) for comparison with the absolute moisture content of the tissue.

The amount of water which was expressed naturally was highly correlated with the amount in the tissue. This correlation was not perfect, however, a larger amount of water remaining in the blade tissue (20 to 27 cc.) than in the stem tissue (8 to 10 cc.). In the blade tissue, moreover, the absolute amount left after pressure extraction was larger in the samples obtained later in the season and in which the original moisture contents were less.

SOLIDS AND SUGARS IN SUCCESSIVE PORTIONS OF SAP

If successive portions of sap expressed from a given sample of tissue have the same composition with respect to certain constituents, the sap remaining in the press cake may be assumed also to have this composition. The total solids, sucrose, and free reducing sugars were determined in suc-

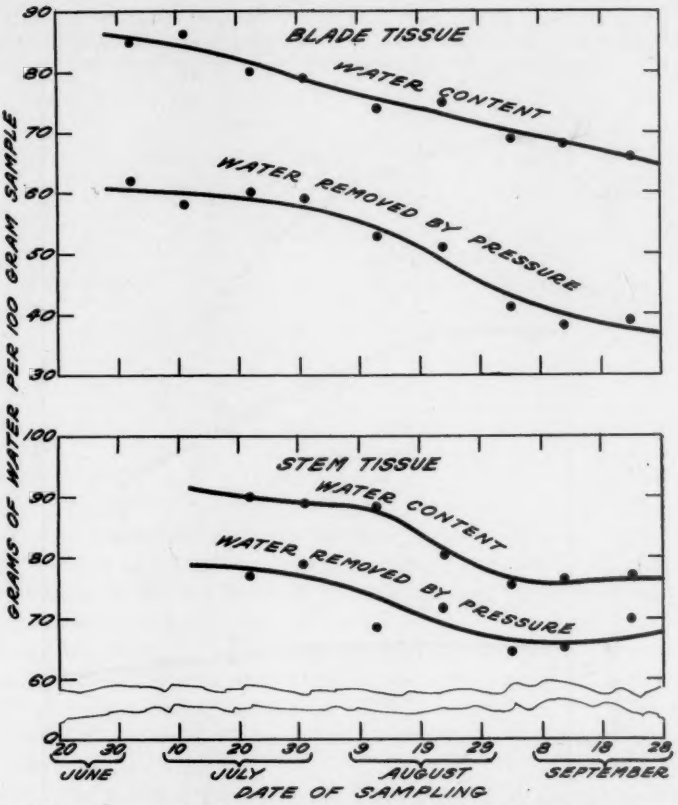

FIG. 2. The water content and the amount of water removed by pressure, from blade and from stem tissue of Burr-Leaming corn, summer 1929.

cessive portions of sap from different tissues after grinding only and after mincing. The data from a representative experiment are shown in table III.

The solids content decreased in the successive portions of sap from all samples. The decrease was greater with blade than with stem tissue, and greater with minced than with ground tissue. The maximum decrease was from 6.2 per cent. of solids in the first portion to 4.2 per cent. in the last portion of sap from minced blade tissue. The corresponding decrease for the ground tissue was from 11.1 to 9.1 per cent. The differences with the stem tissue were similar though usually smaller.

The percentages of sugars were practically the same in the successive portions from ground tissue, whether stem or blade. With the minced

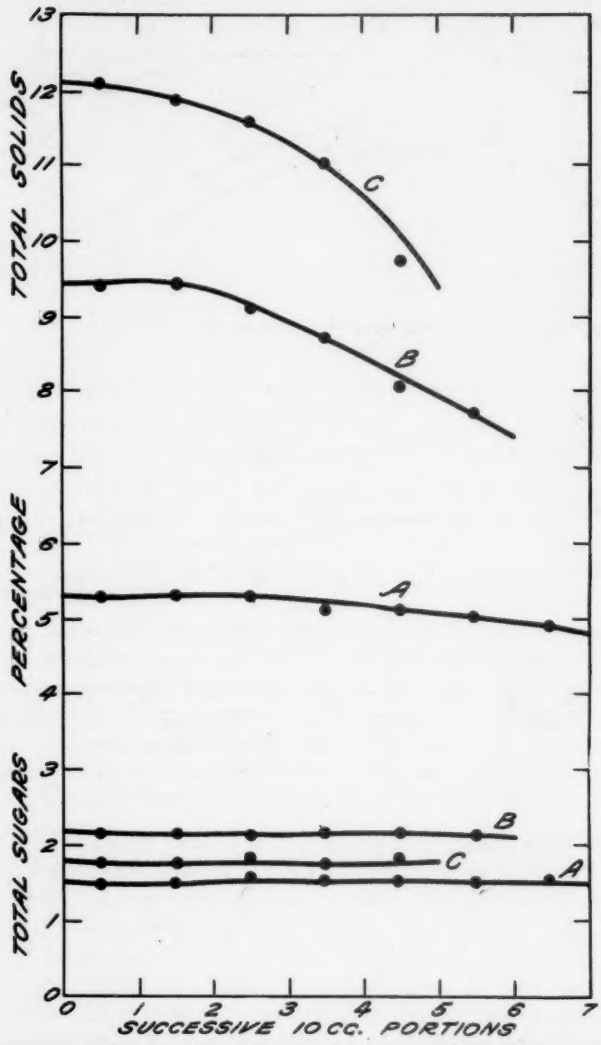

FIG. 3. Percentage of total solids and total sugars in successive 10-cc. portions of sap from blade tissue of Burr-Leaming corn. A = 86 per cent. moisture, B = 74 per cent. moisture, C = 70 per cent. moisture.

TABLE III

PERCENTAGES OF TOTAL SOLIDS AND TOTAL SUGARS, IN SUCCESSIVE 10-CC. PORTIONS OF EXPRESSED SAP OF DIFFERENT CORN TISSUES AFTER DIFFERENT TREATMENTS

PORTION	GROUND				MINCED			
	STEM		BLADE		STEM		BLADE	
	SOLIDS	SUGARS	SOLIDS	SUGARS	SOLIDS	SUGARS	SOLIDS	SUGARS
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
1	12.9	12.1	11.1	2.9	9.9	10.1	6.2	2.4
2	12.9	12.2	11.3	2.9	9.9	10.0	4.2	1.1
3	12.8	12.1	10.5	2.8	9.3	9.1	.....	.....
4	12.8	12.1	10.3	2.9	8.7	8.0	.....	.....
5	12.8	12.3	9.6	2.7	7.9	7.5	.....	.....
6	12.8	12.4	9.1	2.9	.....	.....	.....	.....
7	12.2	12.2	.....	.....	.....	.....	.....	.....
Last drop	11.8	.....	8.5	.....	7.5	.....	4.0	.....
Duplicate 100-gm. sample	12.9	12.1	10.4	2.9	9.4	9.3	5.2	1.8

tissues, the sugars decreased in the successive portions similarly to the solids. Grinding the tissue resulted not only in obtaining larger percentages of solids in the sap but also in obtaining sap having the same sugar content in successive portions.

The total solids and sugars in successive portions of sap from stem and from blade tissues of corn after grinding were determined at three different stages of growth during the season. The percentages in the blade tissues, containing 86, 74, and 70 per cent. of moisture respectively, are shown graphically in figure 3.

The total solids decreased with the successive portions, the decrease being the largest for the drier (more advanced) tissue and least for the tissue containing the most moisture. Similar differences, but much smaller ones, were found in the successive portions of sap from stem tissue. The total sugar content was the same in the successive portions of sap for both kinds of tissue at all stages of development.

RELATION BETWEEN THE SUGAR CONTENT OF SAP AND TISSUE

The sugar content of successive portions of sap expressed from ground tissue was the same for both kinds of tissue and for three stages of development. It seemed probable, therefore, that the sugar content of the tissue could be calculated with more or less accuracy from the sugar content of the expressed sap and the moisture content of the tissue.

During a seasonal study of the distribution of sugars in the corn plant, 58 pairs of samples for sugar determinations were taken. The sugar con-

tent of one sample of each pair was determined by the "standard" alcoholic extraction method and the sugar content of the other was estimated from the sap analysis and the moisture content. In calculating the sugar content of the tissue from sap analysis, no correction was made for the specific gravity and total solids content of the sap. Subsequent data have shown that without this correction the tendency is for the sugar content of the tissue estimated from sap analysis to be slightly low. The samples comprised blade, sheath, and stem tissues from Burr-Leaming and Medina Pride corn, during the period from July 2 to September 24.

The average sugar content of the three tissues from each kind of corn as determined by the two methods is shown in table IV, and the detailed comparison for Burr-Leaming tissues is shown graphically in fig. 4.

TABLE IV

PERCENTAGE SUGAR CONTENT OF CORN TISSUE (1) DETERMINED BY THE STANDARD METHOD, AND (2) CALCULATED FROM THE SUGARS IN EXPRESSED SAP AND THE MOISTURE IN THE TISSUE

TISSUE	BURR-LEAMING				MEDINA PRIDE			
	NUMBER OF SAMPLES	METHOD		DIFFERENCE	NUMBER OF SAMPLES	METHOD		DIFFERENCE
		STANDARD	SAP			STANDARD	SAP	
		per cent.	*per cent.*					
Blade	9	2.14	2.06	−0.08	9	2.11	2.26	+0.15
Sheath	17	4.79	4.50	−0.29	7	4.56	4.40	−0.16
Stem	14	7.82	7.71	−0.11	12	7.98	7.42	−0.56
All	30	5.41	5.27	−0.14	28	5.24	5.00	−0.24
All, both varieties	58	5.33	5.14	−0.19				

The maximum difference between methods was a deficiency of 0.9 per cent. in stem tissue of Medina Pride having a sugar content of 9.1 per cent. (standard method), or a difference of 10 per cent. On the other hand, a sample of blade tissue of the same variety, containing 2.5 per cent. of sugar by the standard method, was estimated as containing 3.2 per cent. by the sap method. The difference here is smaller absolutely but larger relatively, being 28 per cent. of the total. In general, the sugar content indicated by the sap method tended to be lower, the mean difference for the 58 pairs of samples being -0.19 ± 0.034 per cent.

As an additional criterion, duplicate samples of tissue corresponding to 12 of these 58 were analyzed by the standard method and the values compared with the original determinations. These samples comprised different tissues at different stages of growth with a range in sugar content of from 2.68 to 10.50 per cent. The average difference between the paired samples

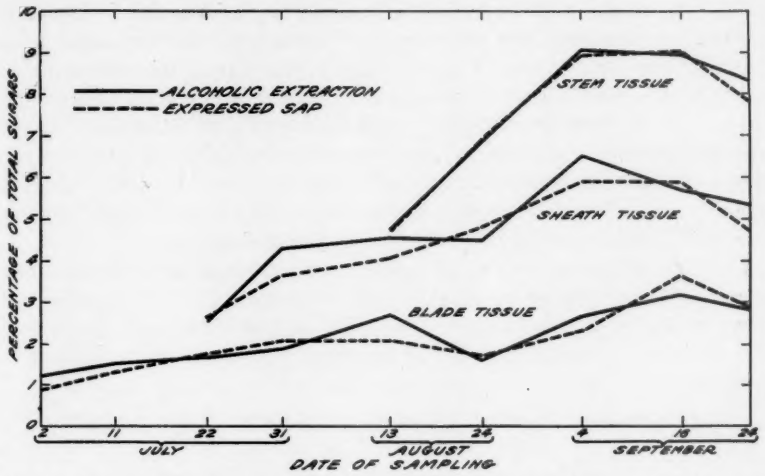

FIG. 4. Percentage of total sugars in blade, sheath, and stem tissue of Burr-Leaming corn obtained by the alcoholic extraction of the tissue and by the expressed sap method.

was 0.26 ± 0.072. The results with the two methods accordingly were no more inconsistent for 58 samples than were those by the standard method for 12 samples, and the difference of -0.19 ± 0.034, though significant statistically, must be considered unimportant in a comparison of the two methods.

The agreement between methods over the entire range of the 58 samples is measured best by the coefficient of correlation between the corresponding values. This coefficient is 0.9913, indicating excellent agreement, with only 1.73 per cent. of the variation (as variance) independent. The data indicate, then, that the sugar content of corn tissues may be estimated from the analysis of sap expressed as described, with little or no error in excess of that to be expected from duplicate samples analyzed by the standard method.

Summary

1. The amount of sap expressed from corn tissue under a uniform pressure and with a uniform time for draining the cage was influenced by the preliminary treatment of the tissue, by the size of sample, and by the kind of tissue and its moisture content as affected by its stage of development.

2. The total solids content differed in successive portions of sap expressed from corn tissue, the size of the differences varying with the treatment preliminary to expression, the kind of tissue and its moisture content.

3. The sucrose and free reducing sugars, reported together here as total sugars, were constant for successive portions of sap expressed after grinding the tissue, but decreased in successive portions from minced tissue.

4. The percentages of total sugars in three kinds of fresh corn tissue from two varieties, at different stages of growth, as determined by the standard method and from the sap expressed after grinding, were in excellent agreement, the coefficient of correlation being 0.9913. The values by the two methods were in as good agreement as those for 12 pairs of samples for which sugars were determined by the standard method.

5. Expressing the sap from a 100-gram sample of ground fresh corn tissue under a pressure of 5,000 pounds per square inch, at the same time forcing the sap through a filter, and allowing a uniform time (5 minutes) for draining, provided samples of sap that were satisfactory for certain physical and chemical examinations.

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EFFECT OF PETROLEUM OILS ON THE RESPIRATION OF BEAN LEAVES

JESSE GREEN AND ARNOLD H. JOHNSON

(WITH FIVE FIGURES)

The injurious effect of petroleum oils on fruit trees is a serious objection to their use as insecticides. The damage appears in a great variety of ways (**10**). Trees sprayed in dormant stage show injury during the following spring as a wrinkling or withering of the bark and retardation of leaf formation. The damage to sprayed leaves generally appears as oil soaked spots or a more acute drying or burning of the leaf.

The exact physiological actions accompanying injury from oil are not well known. The problem appears to be divided into two major possibilities; plugging of stomata and poisoning of cells. In either case respiration should be affected. It has been the purpose of this work to determine the change in rate of respiration of plants which have been sprayed with oil.

Mechanical injury has been shown by RICHARDS (**9**) and others (**3**) to increase the rate of respiration. Toxic and anesthetic materials at certain concentrations are also expected to increase the respiration of plants (**2, 8**).

KNIGHT, CHAMBERLIN and SAMUELS (**6**) have determined the effect of a viscous, highly refined oil in the rate of respiration of citrus trees. Their results show that respiration is greatly increased and remains abnormally high for a long period of time after treatment with this type of oil.

Believing that respiration is generally increased when a plant is injured, an attempt has been made in this study to show the injurious effect on the respiration of bean leaves by the different types of oil used as sprays.

Methods

PLANTS

Dandelion and potato leaves were first used, but later bean leaves were found to give more uniform results. Three- to six-gram samples of bean leaves were taken and the stems placed in water as soon as they were cut. Four samples were used for checks and four were sprayed with oil.

SPRAYING

The spraying was done with an atomizer operated with compressed air (figure 1). It was calibrated before each determination to deliver a definite

amount of oil. This was done by spraying weighed filter papers and then weighing after spraying to determine the amount of oil delivered. The leaves were then sprayed with the same nozzle adjustment, temperature and air pressure. The time of spraying the leaves was adjusted to apply 10 milligrams of oil on a circular area 7 centimeters in diameter, which was the size of the filter-paper used for calibration.

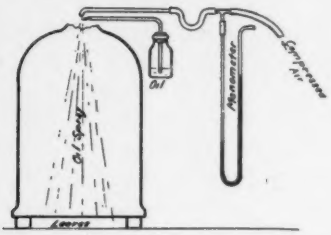

FIG. 1. Apparatus for spraying leaves.

Considerable preliminary work was done on the effect of the amount of oil within the range between very light spraying and dipping the leaves in oils. The amount finally selected gave a visible coating of oil on the leaves and, when calculated, is 0.26 milligram of oil per square centimeter.

OILS

Petroleum oils that are used for spraying are usually of the lubricating type. Nine kinds of oils were selected for experimental use. They had widely varying characteristics, as is shown in table I. The oils were ap-

TABLE I

OILS USED IN RESPIRATION EXPERIMENTS

No.	MANUFACTURER	MANUFACTURER'S NO.	TRADE NAME	SULPHONATABLE RESIDUE	VISCOSITY
				per cent.	*deg.*
1	Standard	13604R	100 Pale	15	100–110
2	Standard	13605R	Calol red engine	44	220
3	Standard	13606R	Oronite technical	5	100
4	Standard	13607R	Mineral seal	10	50
5	Standard	14510R		35	110
10	Standard	13240R		39.7	140
13	Shell	R–L–99		18.3	72
21	Shell	106		14.4*	53
24	Sonneborn & Sons		Amelie	none	67

* SO_2 treated.

plied in the pure state. Emulsions were not used because they would have introduced too many variable factors for this problem.

METHOD OF MEASURING RESPIRATION

A simple method for measuring respiration was devised, following the plan used by LUND (7). After the leaves had been sprayed they were fastened, by means of a rubber band, to a stopper on the tube *a*, figure 2, and were placed in a one-half gallon Mason jar made of white glass. The tube *a* extended downwards about one-half the depth of the jar, and had another stopper on the lower end to keep the leaves apart.

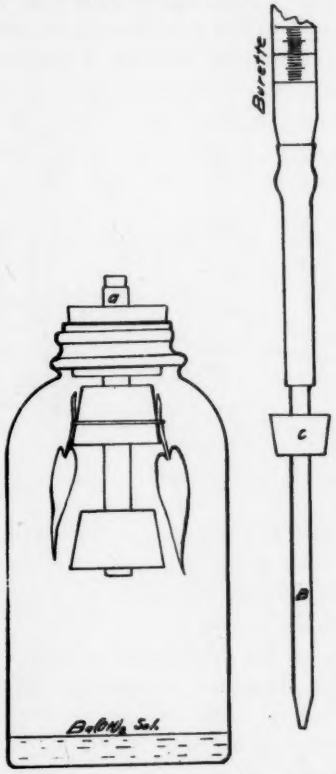

FIG. 2. Respiration jar and burette for measuring and titrating $Ba(OH)_2$ solution.

A titration set was prepared with long tips *B* that would reach down through the tube *a*. One burette was used for adding the $Ba(OH)_2$ solution which absorbed the CO_2 from the plants, and another for titrating the excess $Ba(OH)_2$ with HCl at regular intervals. The tips were selected so as

to fit the tube *a* closely, which was kept stoppered except during titrations. The tip had a rubber stopper *c* near the top which was held down tightly against the tube *a* while titrations were being made to exclude as much outside air as possible.

When the leaves were in place, 25 to 50 cc. of approximately 0.010 N $Ba(OH)_2$ were measured into the jar. Two blank jars without leaves were prepared in exactly the same way, and all were placed on a shaker in an asbestos-lined, constant temperature box maintained at 28° C., figure 3. The shaker was operated with a small motor with a fan on its shaft for circulating the air in the constant temperature box. A very slight shaking motion was sufficient to rock the solution and to circulate the gases in the respiration jars, thus continually exposing a new surface of $Ba(OH)_2$ to the CO_2.

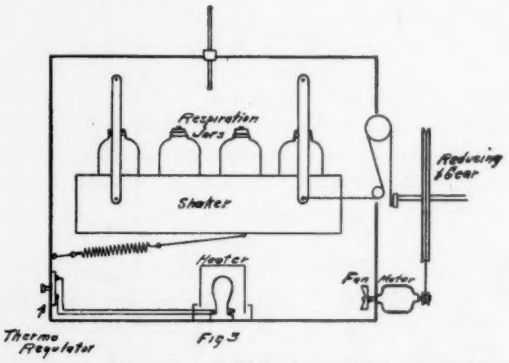

FIG. 3. Respiration jars on shaker in constant temperature box.

Titrations were made at two-hour intervals with exactly 0.005 N HCl. Phenolphthalein was used as the indicator. In the preliminary work the indicator was added in alcoholic solution in the ordinary way, but, to avoid the effect of the alcoholic vapors on the leaves, the phenolphthalein was later placed directly in the standard $Ba(OH)_2$ solution. When the $Ba(OH)_2$ solution was prepared a standard aqueous solution of the phenolphthalein was added until the desired color was obtained. The effect of a small amount of alcoholic vapor in the closed system of the respiration jars was not known, but the probabilities are that it is considerable.

In calculating the results the titrations of the blank jars were subtracted from those of the samples, the differences representing the CO_2 given off from the leaves. One cc. of 0.005 N HCl equals very nearly 0.11 mg. of CO_2.

Discussion

In the closed system for respiration one of the first questions that arises is the effect of the change in concentration of gases; however, the change is very small. The oxygen in a one-half gallon jar can be decreased only very slightly by 3 to 6 grams of leaves in 10 hours. According to KOSTYCHEV (**5**) the concentration of oxygen has very little effect on the respiratory process as long as some oxygen is present. It is stated that a reduction to one-half the normal concentration of oxygen does not change the rate of respiration.

The concentration of CO_2 is kept constant in the closed system, as the moving $Ba(OH)_2$ absorbs it as fast as it is formed. The concentration of water vapor may be important. KELLEY (**4**) states that a high humidity favors oil damage, while on the other hand YOUNG (**10**) finds that in field work the greatest damage from oil occurs when trees are suffering from drought. In the method used, the leaves were in a saturated atmosphere and seemed to go through the ten-hour tests without any apparent injury.

The effects of light and temperature were reduced to a minimum. The constant temperature box was heated with an electric light bulb, automatically controlled. This was covered to eliminate the effect of light in the box. The leaves were in total darkness except for the 3 to 5 minutes required to make titrations at the two-hour periods. Any effect of light and temperature changes was very nearly the same on all the samples.

The age and vitality of the leaves were found to have a great influence on their respiration. Young leaves respire at a more rapid rate than older ones, per gram of leaf. For this reason much care was used in selecting the samples. The curves show that in some determinations the average rate of respiration was much higher than in others even though samples were selected from plants of very nearly the same age.

Because of the variability of samples, several leaves were used in each test and four checks were always run against four treated samples. At the conclusion of the work it was found that even this number was not sufficient. A calculation of the variability of two check samples against two other checks of the same series, showed that the average variability for a group of 17 determinations was 7.5 per cent. It is apparent that the variability of four checks compared with four others, as in the case of the experiments, would be less, probably of the order of one-half to one-third less, making the actual error of the work 4 to 6 per cent.

The variation of the treated samples was much greater than the normals. The average variation of the treated samples, when computed in the same way, was 11.5 per cent., which indicates that respiration was greatly affected by oil.

In the beginning, increasing amounts of oil were expected to gradually accelerate respiration until a maximum should be reached, after which more oil would reduce the rate of respiration.

A series of experiments, using kerosene, was performed to show this effect, because kerosene was expected to be more injurious than lubricating oils. Four experiments were conducted in which the amount of kerosene was doubled each time and finally one set of samples was dipped in kerosene. The results are shown in table II and in figure 5, curves nos. 18, 19, 20, 21 and 22. The respiration rate increased quite regularly with increasing amounts of oil until a maximum was reached after which more oil caused decreased respiration. When the leaves were dipped in oil their respiration dropped to 52.2 per cent. of the normal rate.

TABLE II

RESPIRATION OF NORMAL BEAN LEAVES AND BEAN LEAVES SPRAYED OR DIPPED IN KEROSENE

CURVE NO.	TREATMENT	CO_2 GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO_2 FOR 8 HOURS	DIFFERENCE	GAIN	LOSS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent.*	*per cent.*
18	Sprayed 1 min.	1.34	1.36	1.57	1.43	1.40	7.10			
18	Normal	0.69	1.13	1.24	1.05	1.02	5.13	1.97	38.4	
19	Sprayed 2 min.	1.23	1.37	1.58	1.47	1.51	7.16			
19	Normal	1.01	1.11	1.22	1.08	1.04	5.46	1.70	31.1	
20	Sprayed 4 min.	1.28	1.66	1.85	1.60	1.54	7.93			
20	Normal	1.14	1.33	1.28	1.04	0.91	5.70	2.23	39.1	
21	Sprayed 8 min.	1.34	1.65	1.63	1.29	1.34	7.25			
21	Normal	1.14	1.45	1.17	1.05	1.04	5.85	1.40	23.9	
22	Dipped in kerosene	1.15	0.62	0.38	0.47	0.46	3.08			
22	Normal	1.73	1.59	1.25	1.32	1.28	7.17	4.09		57.0

The lubricating oils used may be divided into two classes: light colored, and dark colored. The dark colored oils were usually more viscous and contained greater amounts of sulphonatable residue than light colored oils.

The term sulphonatable residue designates that portion of an oil that can be extracted with strong sulphuric acid under strictly analytical specifications. It consists largely of unsaturated hydrocarbons, sulphur, nitrogen and aromatic compounds. There is evidence that some of these sulphonatable compounds are toxic to plants. GRAY and DE ONG (1) have shown from field tests that injury increases very closely with the percentage

of sulphonatable residue. Perhaps the unsaturated hydrocarbons are responsible for the trouble, but many of the compounds in the sulphonatable residue are unstable and may have deleterious effects on living plants.

A comparison of the effect of oils of low sulphonatable residue, figures 4, 5, oils nos. 3, 4 and 24, with those of high sulphonatable residue,

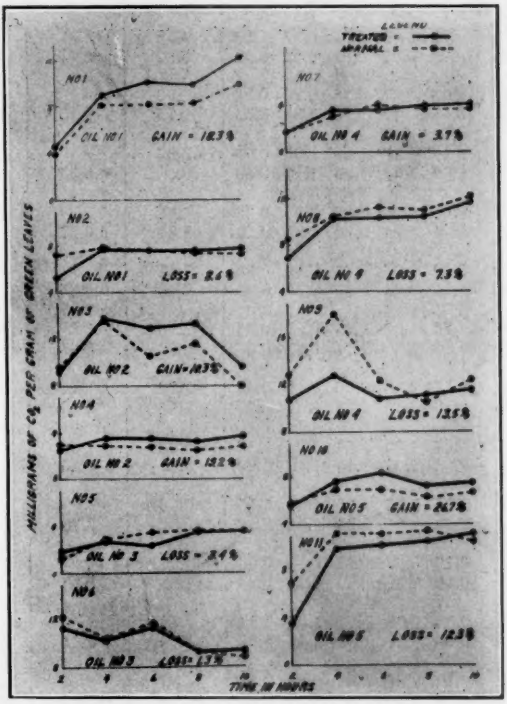

FIG. 4. Curves showing the effect of oil on the respiration of bean leaves.

nos. 2, 5 and 13, show that the former group caused an average loss of 5.8 per cent. in rate of respiration in 7 determinations, while the latter caused an average gain of 8.1 per cent. in 6 determinations. By arranging all of the light colored oils of less than 16 per cent. sulphonatable residue in one group and the darker colored oils of more than 16 per cent. sulphonatable residue in another group, a similar difference is shown. In 8 determinations the light colored oils caused an average loss of 5.0 per cent. in rate of respiration, while, in 9 determinations, the dark oils caused an average gain of 7.5 per cent. The analytical results are shown in table III and are graphically represented in figures 4 and 5, curves 1 to 17.

TABLE III

RESPIRATION OF OIL SPRAYED AND NORMAL BEAN LEAVES

CURVE NO.	OIL NO.	CO_2 GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO_2 FOR 8 HOURS	DIFFERENCE	GAIN	LOSS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent.*	*per cent.*
1	1 Sprayed	0.46	0.90	1.01	0.99	1.23	4.59			
1	Normal	0.39	0.81	0.81	0.82	0.99	3.82	0.77	18.3	
2	1 Sprayed	0.13	0.37	0.36	0.36	0.38	1.60			
2	Normal	0.33	0.39	0.36	0.34	0.33	1.75	−0.15		8.6
3	2 Sprayed	0.90	1.38	1.29	1.33	0.96	5.86			
3	Normal	0.95	1.35	1.05	1.16	0.80	5.31	0.55	10.3	
4	2 Sprayed	0.25	0.36	0.36	0.33	0.38	1.68			
4	Normal	0.30	0.29	0.28	0.25	0.29	1.41	0.27	19.2	
5	3 Sprayed	0.20	0.27	0.23	0.35	0.36	1.41			
5	Normal	0.10	0.28	0.35	0.37	0.36	1.46	−0.05		3.4
6	3 Sprayed	1.15	1.02	1.13	0.92	0.94	5.16			
6	Normal	1.22	1.04	1.17	0.92	0.88	5.23	−0.07		1.3
7	4 Sprayed	0.16	0.36	0.36	0.40	0.42	1.70			
7	Normal	0.16	0.30	0.42	0.37	0.39	1.64	0.06	3.7	
8	4 Sprayed	0.68	1.03	1.04	1.05	1.18	4.98			
8	Normal	0.84	1.05	1.13	1.12	1.23	5.37	−0.39		7.3
9	4 Sprayed	1.07	1.28	1.07	1.11	1.15	5.68			
9	Normal	1.23	1.81	1.23	1.05	1.25	6.57	−0.89		13.5
10	5 Sprayed	0.16	0.37	0.44	0.33	0.36	1.66			
10	Normal	0.19	0.30	0.30	0.24	0.28	1.31	0.35	26.7	
11	5 Sprayed	0.31	0.99	1.02	1.06	1.12	4.50			
11	Normal	0.68	1.12	1.12	1.15	1.06	5.13	−0.63		12.3
12	5 Sprayed	0.70	0.86	1.02	0.89	0.88	4.35			
12	Normal	0.70	0.90	1.00	0.90	0.89	4.39	−0.04		0.9
13	10 Sprayed	0.48	0.72	0.78	0.69	0.66	3.33			
13	Normal	0.46	0.67	0.69	0.61	0.62	3.05	0.28	9.2	
14	13 Sprayed	0.29	0.90	1.08	0.97	0.97	4.21			
14	Normal	0.30	0.91	1.05	0.90	0.83	3.99	0.22	5.5	
15	21 Sprayed	0.72	1.05	1.00	0.93	0.79	4.49			
15	Normal	0.69	1.02	0.99	0.94	0.83	4.47	0.02	0.4	
16	24 Sprayed	0.72	0.96	0.82	0.86	0.71	4.07			
16	Normal	0.71	1.04	0.88	1.02	0.78	4.43	−0.36		8.1
17	24 Sprayed	0.56	0.81	0.91	0.73	0.84	3.85			
17	Normal	0.64	0.96	1.04	0.80	0.88	4.32	−0.47		10.9
		Total							93.3	66.3

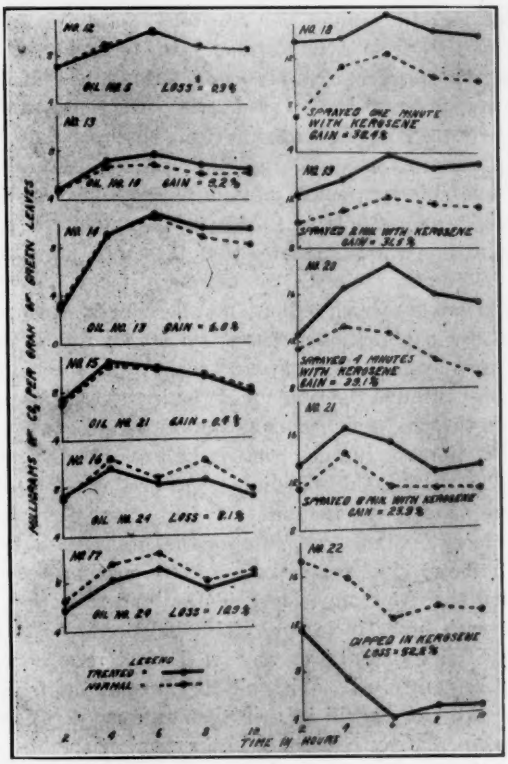
MILLIGRAMS OF CO_2 PER GRAM OF GREEN LEAVES
TIME IN HOURS
LEGEND
TREATED
NORMAL
OIL NO. 5 LOSS = 2.9%
OIL NO. 10 GAIN = 2.2%
OIL NO. 13 GAIN = 6.0%
OIL NO. 21 GAIN = 0.9%
OIL NO. 24 LOSS = 9.1%
OIL NO. 24 LOSS = 10.9%
SPRAYED ONE MINUTE WITH KEROSENE GAIN = 32.4%
SPRAYED 2 MIN. WITH KEROSENE GAIN = 36.8%
SPRAYED 4 MINUTES WITH KEROSENE GAIN = 38.1%
SPRAYED 8 MIN. WITH KEROSENE GAIN = 25.9%
DIPPED IN KEROSENE LOSS = 92.8%

FIG. 5. Left, effect of lubricating oil on the respiration of bean leaves. Right, effect of varying amounts of kerosene on the respiration of bean leaves.

This evidence does not prove that the difference was due only to sulphonatable residue. There is some evidence that sulphonatable residues alone increase respiration. Oil no. 3 is a white oil with a sulphonatable residue of 5 per cent. and a viscosity of 100. Oil no. 5 is a yellow oil with 35 per cent. sulphonatable residue and a viscosity of 110. The difference of viscosity is small and the difference in sulphonatable residue is comparatively large. For 2 determinations oil no. 3 caused an average loss of 2.4 per cent. in CO_2 respired and for 3 determinations oil no. 5 caused an average gain of 4.3 per cent.

The indications are that the sulphonatable residue of an oil causes an increase in the rate of respiration of bean leaves and that the more highly refined oils cause a decrease in the rate. Satisfactory explanations for

these two actions are difficult. The reactions leading up to the taking in or giving off of CO_2 from plants are very involved but in their simplest expression may be considered mainly as oxidation and reduction reactions.

An increase in the rate of CO_2 production over the normal is spoken of in this work as increased respiration. As it has been shown that the oils with high sulphonatable residues cause an increase in respiration it follows that the sulphonatable compounds must in some way favor oxidation in the plant. The light colored oils with low sulphonatable residue cause a decrease in respiration which indicates that these oils have favored carbon reduction or the greater use of CO_2 in the plant itself.

The exact means by which oxidation and reduction are promoted is as yet too difficult for explanation. These two actions are, no doubt, affected in a physical way by the oils whenever there is any interference with the exchange of gases through the stomata of the leaves. A change in the rate of flow of gases through their regular channels would cause changes in the concentration of the reacting material in the leaves at the points of action and may thus have a decided influence on the products formed. However, the chemical action of the compounds in the oil on the plant juices and cells is perhaps the more fundamental cause for changes in the rates of oxidation or reduction followed by changing rates of CO_2 production.

In any event the two tendencies, oxidation and reduction, are well balanced against each other in leaves. This balance is sustained by many factors. It was found that at least some of the factors were not even known and could not be controlled. With all the precautions taken to regulate temperature, light, etc., it will be seen that on some occasions either type of oil caused an increase or a decrease in the respiration rate.

In the case of oil no. 4 (a light oil of low sulphonatable residue) 3 determinations were made and 1 of these showed an increase, which is contrary to the rule. Also, with oil no. 5 (a dark colored oil of high sulphonatable residue) 3 determinations were made and 2 of these showed decreased respiration, which is decidedly different from the general trend of all the oils of this type. It is obvious that there are variables that are not yet under control and it is only by making a large number of determinations that any significance can be attributed to the results.

Both types of oil when used in high concentrations cause damage in the field (**10**). Oil no. 24 is highly refined and has no sulphonatable residue but when used in high concentrations it has caused injury in orchard tests. It may, however, be said that the oils with small amounts of sulphonatable residue cause less injury to foliage than those containing large amounts (**10**). Although this work was begun with the general impression that increased respiration was a sign of injury the question now arises regarding

the relation of decreased respiration to injury. A positive answer can not now be given but the hypothesis will be advanced that any change in rates of respiration from the normal may be looked upon as the result of injury.

Summary

When bean leaves were sprayed with the dark petroleum oils containing more than 16 per cent. of sulphonatable residues, their rates of respiration were increased. In 9 determinations using five dark colored oils the average increase in CO_2 respired by the sprayed leaves over the normal was 7.5 per cent.

Light colored oils of less than 16 per cent. sulphonatable residue caused a decrease in the rate of respiration of bean leaves. In 8 determinations using four different light colored oils an average loss of 5.0 per cent. in CO_2 respired occurred.

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STRANGULATION OF COTTON ROOTS[1]

J. J. Taubenhaus, W. N. Ezekiel and H. E. Rea

(WITH TWO FIGURES)

In 1923, specimens of a peculiar and, until recently, puzzling cotton root trouble were received. The specimens were of cotton plants with well-developed tops but with extremely shallow and very greatly reduced root systems (fig. 1 *a*). There was no indication of attack by fungi or bacteria, nor of injury by insects, rodents, or farm implements, to account for the shortened roots; yet it was difficult to explain how such large plants could have developed without more extensive root systems. These specimens were from widely separated points in Texas: Mercedes, Eloise, El Campo, and Chillicothe. We have had no further record of this condition on cotton in this state until the past season. However, affected cotton plants were received in 1924 from W. E. Ayres, then Assistant Director of the Delta Branch Experiment Station at Stoneville, Mississippi; from Dr. V. H. Young, of the Arkansas Agricultural Experiment Station; and from Burdette, Arkansas.

During the summer of 1929, many specimens were again received from cotton planters in different parts of the State, including the four places where affected plants had been found in 1923. Affected plants were also collected by the writers from cotton fields near Itasca, Buna, Cameron, Palmer, and Von Ormy. It was possible for the first time to study the root systems of affected plants in place in the field. Cotton growers referred to such cotton plants as "rootless cotton," "flat-foot," "club-foot," and "bumble-foot." These terms are undesirable since the affected plants actually possessed well-developed roots, which, however, were constricted just below ground. The name "root strangulation" appears more appropriate and is suggested. Cotton root strangulation has appeared only sporadically, and is of economic importance only in limited areas. In affected fields, in 1929, losses from dead plants ranged from 3 to 8 per cent. of the stand. Many of the affected plants died early in the season, before the bolls were fully developed, while there were often one to five normal bolls on other plants.

Description

The specimens received during 1923 and 1924, and affected plants studied in the field in 1929, were in general similar in appearance. Some

[1] Published with the approval of the Director as Contribution no. 118, Technical Series, of the Texas Agricultural Experiment Station.

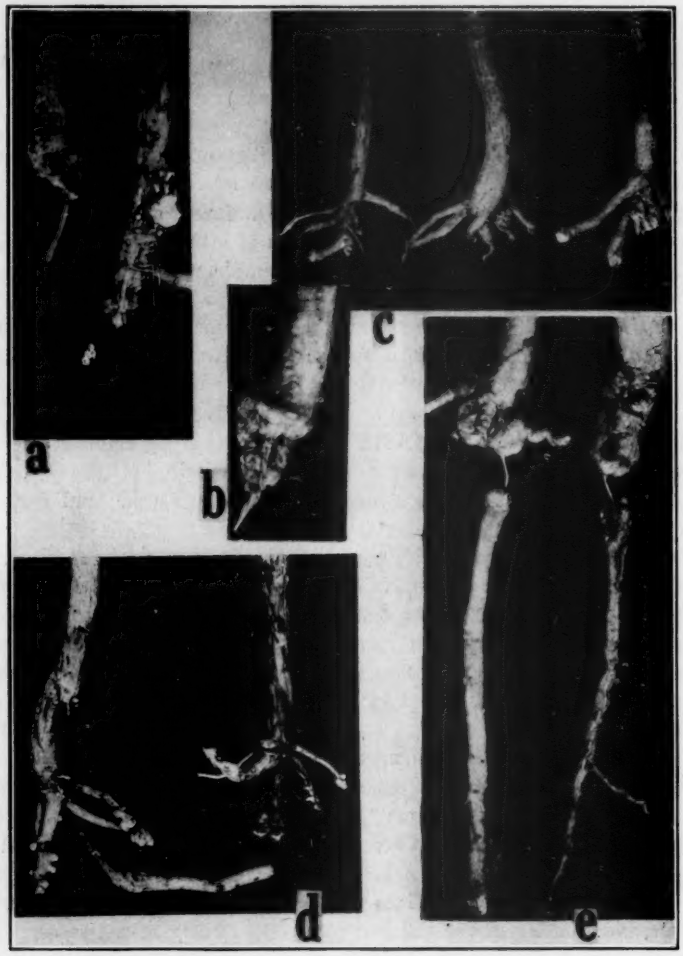

FIG. 1. Root strangulation of cotton, *a* and *b*, upper parts of tap-roots as pulled from the ground, showing roots enlarged just below surface of ground and tapering to needle-like tips; *c*, tap-roots and lateral roots showing strangulation; *d*, strangulated roots with bead-like calluses; and *e*, root systems of strangulated cotton plants as demonstrated by excavations, showing the strangulated areas connecting the upper and lower portions of the roots.

of the plants showed no abnormal symptoms prior to their final wilting and death. Other plants were stunted and noticeably smaller than normal plants. Affected plants are usually noticed first when they wilt suddenly and die without any preliminary yellowing of the foliage. Plants wilting in this way resemble those dying from *Phymatotrichum* root-rot. However, when plants with strangulated roots are pulled, it is found that instead of the decayed roots characteristic of root-rot, the roots are deformed but sound. Immediately below the surface of the ground, the bases of the stems of typically affected plants are considerably enlarged; below these enlarged portions the taproots taper rapidly to blunt points, which terminate in needle-like, fine rootlets. There are sometimes a few short stubby laterals which also taper to blunt points. The short tap-roots and lateral roots are frequently covered with bead-like calluses, which occur either scattered or in clusters. It is to be noted that this description applies only to the upper portions of the root systems which portions come out readily when the plants are pulled. The deeper parts, which are connected to the upper portions only by the constricted regions described below, remain in the ground and appear normal.

The following measurements were obtained from ten affected plants which were pulled from the field. The plants were large and well-grown, ranging from 22 to 43 cm. tall, with an average height of 32 cm. At the surface of the ground, the diameter of the stems averaged 7.2 mm., while at an average of 4 cm. below ground, the diameter averaged 10.0 mm. The average length of the tapered point (that is, the distance from the thickest part of the root to the blunt tip) was only 2.7 cm. The total distance from the surface of the ground to the bottom of the pointed tap-roots thus ranged from 4 to 10 cm., and averaged just under 7 cm. Only half of the plants had any lateral roots; these averaged less than 2.5 cm. per plant. The typical plants, with very much shortened, wedge-like tap-roots, were almost literally "rootless," with the root systems reduced apparently to less than a twentieth the normal size. There were also less severely malformed plants, with longer and more nearly normal lateral roots. The roots of these plants also were characteristically covered with small bead-like calluses. Plants with roots of this sort (fig. 1 *d*) did not wilt so early in the season as was the case with the plants with more severely affected roots (fig. 1 *b*).

By making field excavations of the root systems of affected plants, it was possible to find the probable cause of the trouble.[2] Owing to the protracted dry weather during the summer of 1929, the soil around the plants was found to be unusually hard and compact. The method of excavation was

[2] The writers were assisted by Mr. S. E. WOLFF in excavating plants at the J. W. COFFIN farm at Itasca, Texas.

as follows: Isolated, recently wilted plants were located, and the tops cut off. A deep trench was then dug 12 inches away from the affected plant. With hammer and ice pick, particles of the soil were gently chiseled away until the entire root system of the plant was fully exposed. In some cases, the soil was too dry and hard to enable use of the ice pick. Such plants were removed inside large blocks of soil, which were placed in a wash-tub filled with water. After the hard soil softened, it was then possible to remove the soil from the roots.

It was found that all affected plants, excavated from various fields, actually possessed well-developed root systems. The upper parts of the tap-roots or laterals were, however, separated from the lower parts by constricted areas, often an inch in length, which varied from 0.3 to 0.4 mm. in thickness (fig. 1 *e*). The constricted areas were found in the hardest, subsurface layers of soil; and the more normal lower parts of the roots were found in the less compact soil beneath. It was these needle-like, constricted, connecting portions of the roots which had at first been considered fine rootlets.

Probable cause of cotton root strangulation

This malformation of cotton roots is evidently non-parasitic. There was no evidence of infection or decay in the tissues of the roots, and cultures made from various parts of the roots were uniformly sterile. There was also not the least evidence of injury by implements or insects. It thus appears of significance that the trouble has been found only on sticky, poorly-drained, flat clay soils, and that it occurred only during seasons in which exceptionally heavy rainfall in spring or early summer was followed by prolonged hot, dry weather. Such a sequence of weather conditions occurred both in 1923 and in 1929. In fig. 2, precipitation data are summarized for these years, for one of the fields in which cotton strangulation occurred both years. It will be noted that in 1923, after excessive rainfall in April, there was a drought for four months. Again in 1929, following the record-breaking rains of May, there was very hot, dry weather in June.

A record for one of the farms in which plants were excavated will illustrate the effect of the peculiar weather conditions during 1929 on the cotton crop. The soil was Houston black clay, a deep clay soil which packs and becomes very sticky during rainy weather. Cotton was planted about April 30, and the soil was then thought to be in a fair condition of tilth. By May 4 and 5, the seed came up to a good stand. Frequent beating showers on May 6 and 7 packed the surface of the ground, which was too wet to permit cultivation to break the surface crust, and the rains continued from May 12 to 28. The ground was now thoroughly packed and too soaked for cultivation until June 2. It was not until this time that the ground ap-

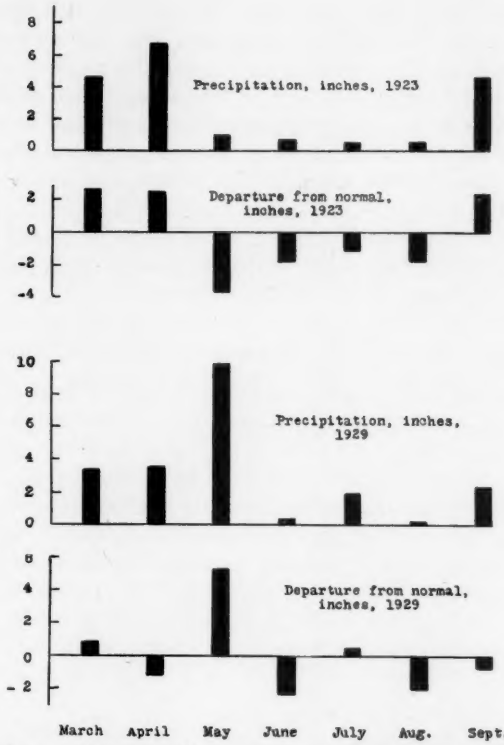

FIG. 2. Rainfall data, 1923 and 1929, averaged from records for Cameron and Valley Junction, Texas, the weather stations nearest to Eloise where root strangulation occurred both years.

peared dry enough for the first cultivation of the season. On account of the hard, packed condition of the soil, the cultivator in many places failed to break the crust. The weather continued hot and dry, and the soil stayed hard and dry, from then until August 17, when affected plants were collected in this field. Plants began to die from root strangulation about the middle of July, and about 5 per cent. had succumbed by August 17.

The following explanation of cotton strangulation thus appears probable. First the seedlings, germinating in the moist soil, developed more or less normal root systems. The early, continued rains packed the surface soil, which was then gradually baked during the hot, dry weather following. The upper portions of the tap-roots and laterals were imprisoned in the hardened, bricklike subsurface layer of soil, and further growth of these

portions of the roots was prevented. Below these imprisoned areas, the roots continued to develop fairly normally. So long as the strangulated areas remained sound, water and food material could still be translocated between roots and stems, although in increasingly inadequate amounts as the plants increased in size. The enlarged, clavate bases of the stems were evidently caused by accumulation of food materials which could not be translocated down to the roots. Final wilting and death of the plants appeared to be directly from lack of water. This may have resulted from inability of the slender connecting tissue to supply sufficient water for unusually hot days, or from death of this thin connecting root in the hard, baked, subsurface soil.

Summary

Root strangulation of cotton has been found in Texas, Arkansas and Mississippi. It seems to occur only in flat, poorly-drained, heavy clay soils, which are compacted by continuous rain or irrigation, and then further hardened, in the absence of cultivation, by continued hot, dry weather. The direct cause of the trouble is apparently that the upper portions of taproots and laterals of the young seedlings are caught early in the season in a subsurface layer of hard, dry clay in which further development is prevented. Affected plants die when the constricted areas in the hot, dry soil are killed, or when the moisture supply which can be transported through the constricted areas becomes too greatly inadequate for the requirements of the plants.

Texas Agricultural Experiment Station,
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TESTS OF CHIBNALL'S METHOD OF EXTRACTION FOR INVESTIGATING WINTER HARDINESS OF PLANTS[1]

W. E. Tottingham, R. G. Shands and E. D. Delwiche[2]

(WITH TEN FIGURES)

Chibnall has published (**2**) a method for extracting separately the vacuolar sap and cytoplasmic fluid in plant cells. It appeared that this procedure might be useful for chemical examination of plant tissues in relation to winter hardiness. The writers were particularly interested in a comparison of the distribution of soluble forms of nitrogen compounds and sugars between the sap and cytoplasmic fluids with the progress of cold weather.

Plant material

A cold resistant variety of wheat was used, varying its resistance by change in the date of sowing. This was Wisconsin Pedigree 2, a strain of the Turkey Red variety. Alfalfa plants were secured from field sowings one year old. A range of hardiness was covered including Grimm as the typically hardy variety, Utah as semi-hardy and Peruvian as non-hardy. Agronomists recognize that winter hardiness of wheat becomes localized in the crown, or base of the stems. In the present investigation the roots were severed and the tissue below the base of the lowest leaf blade taken for analysis. Obviously, this is likely to involve considerable proportions of tissue of lesser pertinence to the investigation, but practical considerations as to the quantity of tissue rendered the grosser sampling necessary. In alfalfa the crown tissue is more limited and less definitely defined than with wheat. Hence, for the present purpose, a top portion of the tap root about 10 inches in length was taken. This was divided into upper and lower sections of about equal length. When the soil became deeply frozen it was necessary to blast, allowing the fragments to thaw at room temperature over night for recovering the plants. It must be admitted that the time elapsed in recovering tissue in this manner might permit considerable changes to occur in its composition. In the earlier samplings of wheat it was found possible to loosen the crowns quickly by playing tepid water over the soil blocks, but the heater available lacked sufficient capacity.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] The writers are indebted to Professors J. G. Dickson, Department of Plant Pathology, and L. F. Graber, Department of Agronomy, for support which made this work possible.

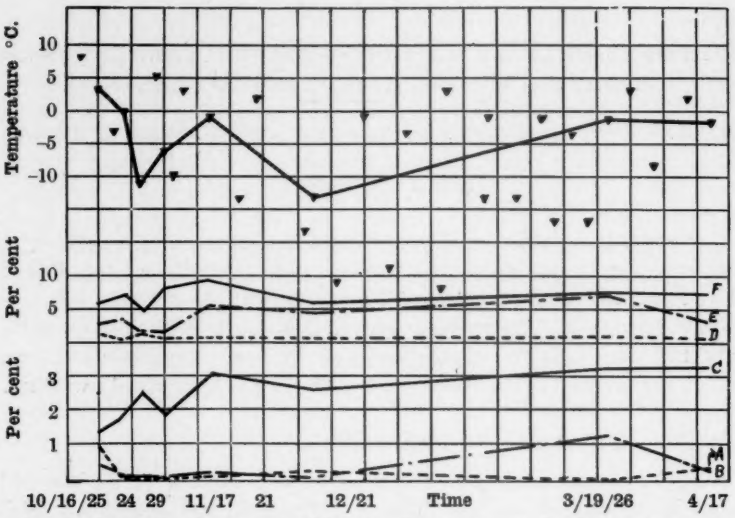

FIG. 1. Relation of sap composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

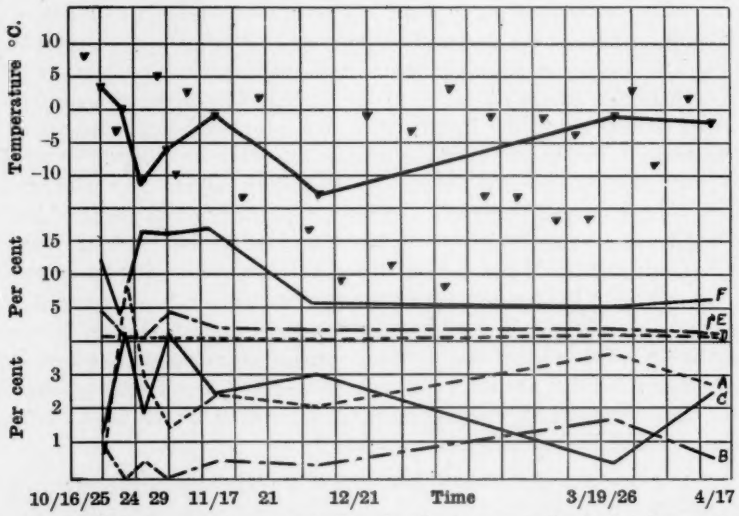

FIG. 2. Relation of extract composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

Analytical procedure

Inasmuch as this is a test of extractive method, rather than a finished metabolic investigation, it seems sufficient to give here only the broader and more pertinent aspects of both the analytical treatment and the resultant data.

The rapidly washed tissue was freed of surface moisture by absorption and chopped to small fragments. In addition to samples for moisture and total nitrogen, a relatively large aliquot (50 to 100 gms.) was taken for extraction. This was inclosed with a little ether for cytolyzing, expressed in a heavy, hand-operated press, and rinsed out with water to remove the vacuolar sap. The residual tissue was ground in a Nixtamal mill with liberal addition of water, pressed and rinsed in cheese cloth and filtered through a layer of paper pulp. In this way the protoplasmic extract was obtained. Care was taken, of course, to maintain uniform conditions throughout the extraction of successive samples. In the analytical examination procedures conformed to recommendations already published (**1**). The results are expressed as percentages of the original dry matter.

Obviously, considerable variation in the analytical data should be expected and only those of major magnitude should be seriously considered. For this reason the results are not given in detail but rather in the generalized form of graphs. On these latter the inverted heat symbols show the frequency and extent of major departures in the minimal temperatures. This practice is based upon HARVEY'S observation (**5**) of hardening efficiency in alternating temperatures.

Interest in the results here presented is conditioned by the possibility that proteins of the protoplasm may either enter into combination with other compounds protective against cold or become favorably altered in the hardening process. Such changes should probably be anticipated less in the sap, but it should be recognized that exchanges of constituents may occur in both directions between the cytoplasm and vacuolar sap.

Discussion

The results for wheat from a date of planting favorable to cold hardiness are depicted in figures 1 to 3, inclusive. In this case the sap protein exhibits marked depression throughout the period of sub-zero temperatures. This loss was compensated by an increase of other than basic nitrogen compounds, which may be assumed with considerable justification to consist largely of simpler peptides and monamino-acids. These changes were accompanied by increases of sugars, and particularly of sucrose, in the early period of lower temperatures.

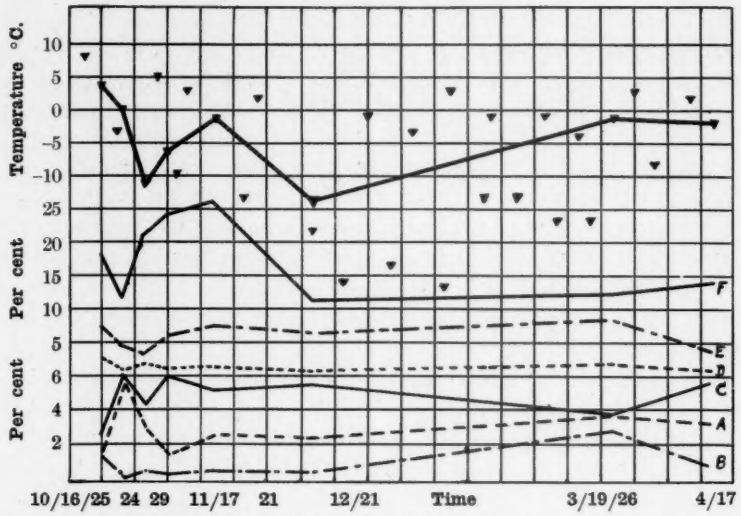

FIG. 3. Relation of total solutes composition in wheat crown to hardening temperatures. Favorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

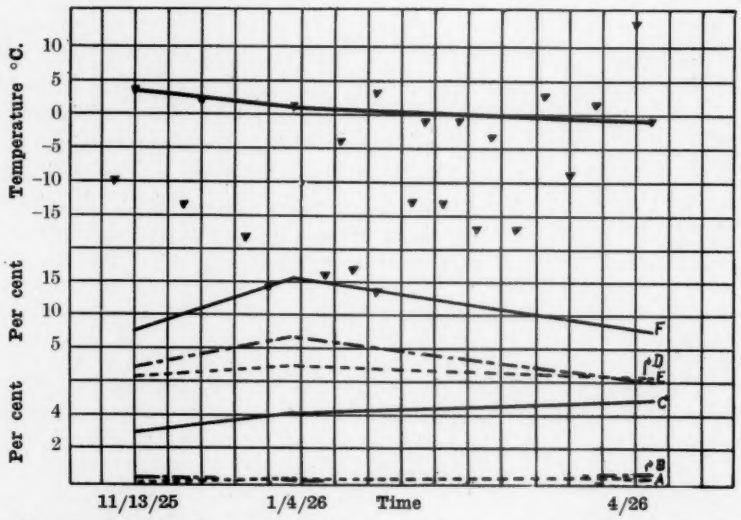

FIG. 4. Relation of sap composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

The protein of the extract appears to have varied sharply, but its general trend was toward increasing with hardiness. However, this relation applies more distinctly to the "rest" fraction, already postulated as involving simpler peptides and monamino-acids. Concomitant with these changes was a marked accumulation of sucrose. This phenomenon is particularly striking. Apparently sucrose was fixed in some manner in the protoplasm or it should be more largely recovered in the sap. This furnishes evidence additional to that of NEWTON (**7**) of protection provided by sucrose against coagulation of protein by freezing. The present indications of hydrolysis of proteins in hardening is in agreement with the early observation of HARVEY (**4**) on cabbage and that of NEWTON (**7**, p. 39) on wheat. No further interpretation is suggested by the combined values of sap and extract.

The results with wheat from the later, unfavorable date of sowing are shown in figures 4 to 6, inclusive. Here the sap displays a continuous increase of the "rest" nitrogen fraction. This change was accompanied by increases of all forms of carbohydrates during the hardening season, sucrose predominating. In the extract the variation of nitrogen compounds was relatively insignificant during the earlier cold weather. During this period a decided increase of reducing sugars was accompanied by a striking decrease of sucrose. These latter changes are conspicuous in the summated results, and are in marked contrast to those occurring in the test with hardier plants.

In the case of alfalfa roots the results are presented in figures 7 to 10, inclusive. The sap shows little orderly change in nitrogen compounds excepting a late increase of the alpha-amino fraction in the Grimm variety. Inspection of the temperature graph indicates that one might not expect hardening to have been far advanced until the last date of sampling. Sugars increased a little in the order of hardiness. In the extract, protein increased appreciably in Grimm but not in the less hardy varieties. A similar trend is shown by the alpha-amino fraction. The sugar curves give no consistent variation but those of the dextrin-starch fraction show declines in the order of hardiness. No further observations are suggested by the summated composition of sap and extract.

Of the insoluble components the variations of protein are too irregular to bear interpretation. The values of this constituent were based on the assumption that all nitrogen remaining in the tissues after extraction was present in proteins. To it is added the protein of sap and extract to give total protein. Decrease of starch in all varieties is one of the most definite effects here observed, being most prominent in Grimm and at an early stage. These observations of increases in sugars and soluble nitrogen compounds

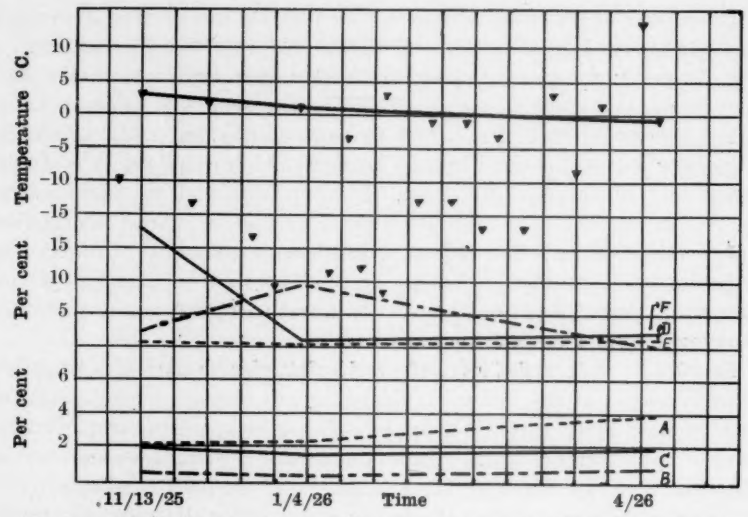

FIG. 5. Relation of extract composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

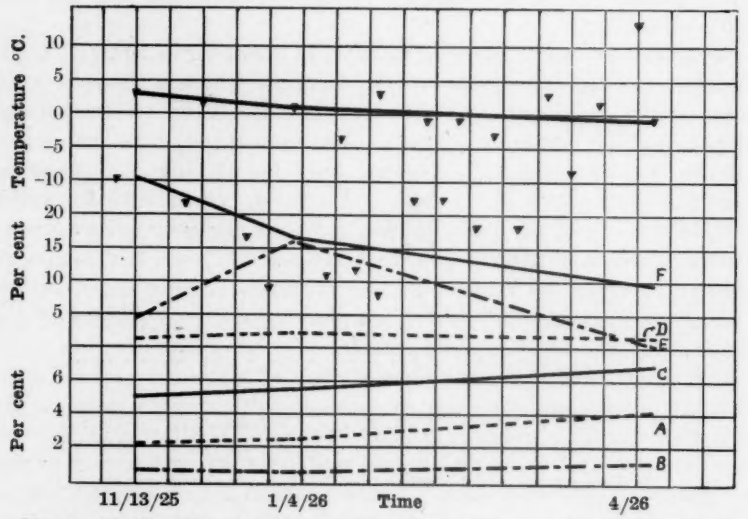

FIG. 6. Relation of total solutes composition in wheat crown to hardening temperatures. Unfavorable sowing date. A = protein, B = basic N x 5, C = rest N x 5, D = pentosan, E = reducing sugars, F = sucrose.

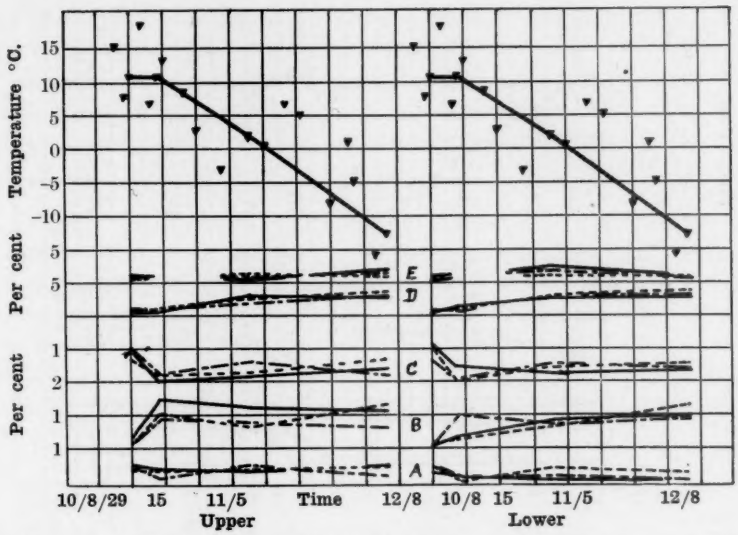

FIG. 7. Relation of sap composition to hardening in alfalfa root. ----= Grimm, ——— = Peruvian, — — — — = Utah. A = protein, B = α-amino N x 5, C = rest N x 5, D = total sugars, E = dextrins and soluble starches.

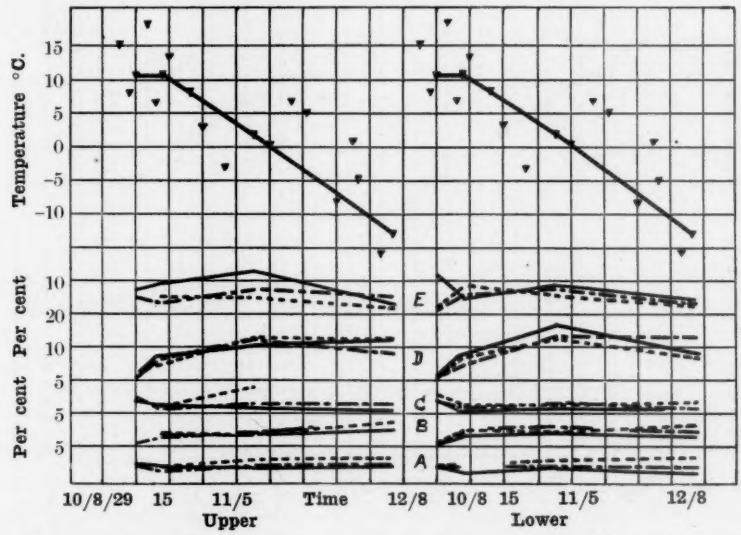

FIG. 8. Relation of extract composition to hardening in alfalfa root. ----= Grimm, ——— = Peruvian, — — — — = Utah. A = protein, B = α-amino N x 5, C = rest N x 5, D = total sugars, E = dextrins and soluble starches.

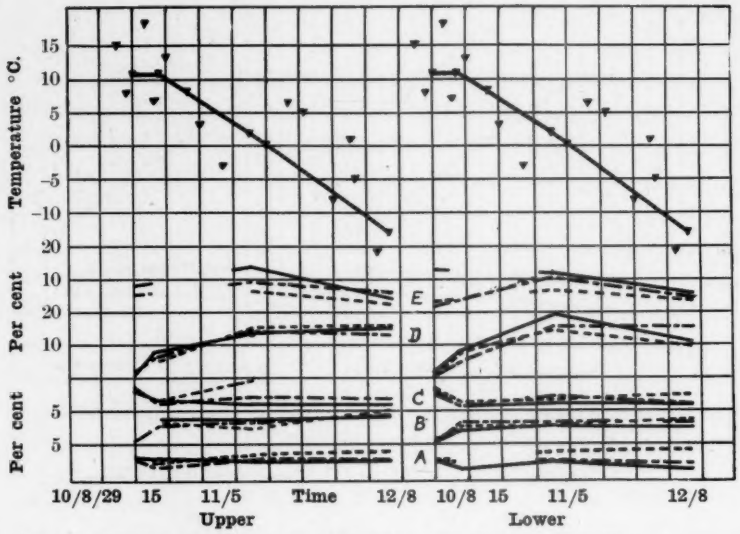

FIG. 9. Relation of total solutes composition to hardening in alfalfa root. ----- = Grimm, ——— = Peruvian, ——— = Utah. A = protein, B = α-amino N x 5, C = rest N x 5, D = total sugars, E = dextrins and soluble starches.

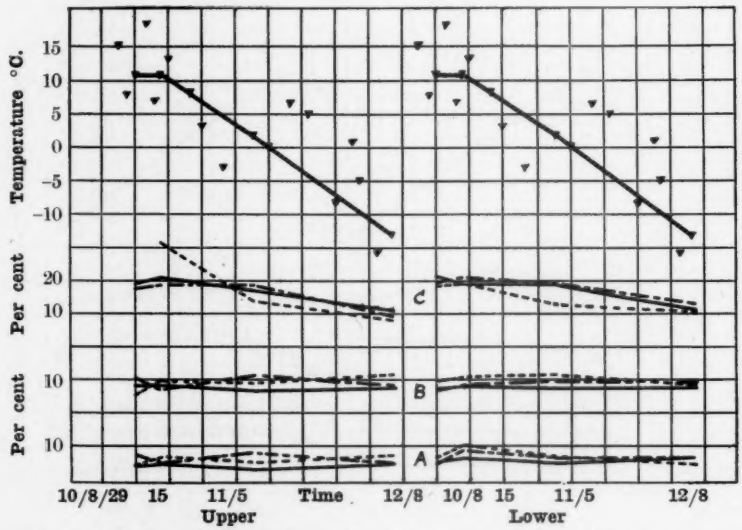

FIG. 10. Relation of insoluble constituents to hardening in alfalfa root. ----- = Grimm, ——— = Peruvian, ——— = Utah. A = protein, B = total protein, C = starch.

and decrease of starch agree with the results of STEINMETZ (**8**) and JANSSEN (**6**).

Some constituents, such as extract protein and insoluble starch, display good agreement between values of upper and lower root. This is not to be expected generally, for the bud and crown tissue included in the upper section might well differentiate its metabolic behavior from that of other root tissue. Numerous irregularities in the data indicate that extensive operations might be necessary according to the procedures here followed to yield conclusive results. If sampling could be localized to the more pertinent crown tissues, as suggested by the observations of STEINMETZ (**8**, p. 10), one might hope for more significant results. Because of our immediate interest in a method of attack upon the hardiness problem developed at this institution (**3**) it is unlikely that we can soon give CHIBNALL'S method the further tests it appears to merit. We therefore present the results of these limited trials on their face value.

Conclusions

Separate chemical analysis of the protoplasmic extract by CHIBNALL'S method of recovery from selected tissues of wheat and alfalfa discloses changes of composition which may be related to the development of cold hardiness. This method of tissue fractionation appears to merit further tests of its usefulness.

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COMPOSITION AND QUALITY OF PENNSYLVANIA CIGAR-LEAF TOBACCO AS RELATED TO FERTILIZER TREATMENT[1, 2]

D. E. Haley, J. B. Longenecker and Otto Olson

The quality of the smoke produced during the normal combustion of a cigar is largely dependent on the chemical composition of the tobacco. The chemical composition of the tobacco may be influenced by many factors, among which is the fertilizer treatment employed by the grower. In order to obtain the best quality cigar-leaf tobacco, it is essential to recognize the requirements and limitations of the growing plant, to insure the presence of the materials deemed necessary, and to guard against those materials that are not only unnecessary but even harmful, so far as quality production is concerned. The individual effects of a fertilizer treatment, however, may be considerably influenced by many environmental conditions, among which is the inherent fertility of the soil itself. For example, the soil of the experimental tobacco plots at Ephrata, Lancaster County, Pennsylvania is extremely high in calcium and quite low in available potassium. This may not hold true for tobacco soils in other sections of the county; hence the optimum fertilizer treatment for the experimental plots may not hold true for other soils in the county.

In former publications (**1, 2**) we have recognized the fact that for quality production of tobacco on our experimental plots we must equalize, if possible, the calcium and potassium content of the plants. In 1928 we planned an experiment having this objective.

Plan of the experiment

Avoiding the use of lime, but not taking into account those factors which might influence the availability of soil calcium, we planned an experiment making potash the most important variable. Ten plots were used, each one-thirtieth of an acre in area. The crop previously grown on these plots was red clover. Five of the plots received manure, with or without additions of fertilizer. The remainder received individual fertilizer treatments, but no manure. The treatments are given in table I.

At the end of the growing season twenty plants from each plot were air-cured. At the end of this period they were dried at room temperature. When dry, the webs of the leaves were ground and kept in sealed receptacles for analysis.

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper no. 505.

[2] This investigation was conducted in cooperation with Dr. W. W. Garner, of the U. S. Bureau of Plant Industry, Office of Plant Nutrition and Tobacco Investigations, and Professor F. D. Gardner, Department of Agronomy of the Pennsylvania State College.

TABLE I

FERTILIZER TREATMENTS OF THE EXPERIMENTAL PLOTS
ACRE BASIS

PLOT NO.	MANURE	COTTONSEED MEAL	PRECIPITATED BONE* PHOSPHATE	NITRATE OF SODA	SULPHATE OF POTASH	CARBONATE OF POTASH	UREA
	tons	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
A–1	10	400	130	125	200	.....	.....
A–2	10	400	130	125	400	.....	.....
A–3	10	400	130	125	600	.....	.....
A–4	10	400	130	125	.....	310	.....
A–5	10	.....	.....	.....	.....	.....	.....
A–6	.....	600	195	187	300	.....	.....
A–7	.....	600	195	187	600	.....	.....
A–8	.....	600	195	187	900	.....	.....
A–9	.....	600	195	187	.....	465	.....
A–10	.....	375	460	187	.....	465	57

* 36 per cent. P_2O_5.

The remaining plants were similarly cured; at the end of this period they were fermented for several months. Uniform samples then were taken for analyses, as mentioned above, and a part of the remainder were made into cigars.

Analyses of the samples were made according to methods as described in previous publications (**1, 2**), with but few modifications. A special effort, however, was made to determine separately the soluble and insoluble portions of the total ash, thus guarding against the possibility of weighing the calcium as the hydrate or carbonate rather than the oxide.

TABLE II

STUDY OF THE CALCIUM, MAGNESIUM, SULPHUR AND POTASSIUM CONTENT OF CURED TOBACCO AS INFLUENCED BY FERTILIZER TREATMENT

PLOT NO.	CaO	MgO	SO_3	K_2O
	per cent.	*per cent.*	*per cent.*	*per cent.*
A–1	7.95	0.84	1.14	2.57
A–2	7.55	0.75	1.16	3.16
A–3	7.45	0.80	1.32	3.35
A–4	7.76	0.81	1.16	3.21
A–5	7.65	0.96	1.18	1.74
A–6	8.09	0.92	1.40	2.81
A–7	6.97	0.82	1.47	3.26
A–8	7.01	0.85	1.68	3.27
A–9	7.23	0.81	1.05	2.84
A–10	7.67	0.85	1.49	2.23

Analytical results

A study of the calcium, magnesium, sulphur, and potassium of the cured samples was made first; the results are given in table II.

This analysis was followed by a study of the soluble and insoluble ash constituents of both the cured and fermented samples, and their alkalinity; the results are given in tables III and IV.

TABLE III

ASH OF THE CURED AND FERMENTED CIGAR-FILLER TOBACCO AS MODIFIED BY FERTILIZER TREATMENT

PLOT NO.	ASH					
	SOLUBLE		INSOLUBLE		TOTAL	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
A–1	3.99	5.49	13.47	10.45	17.46	15.94
A–2	4.64	6.01	11.95	10.63	16.59	16.64
A–3	4.76	6.38	12.02	10.10	16.78	16.48
A–4	4.51	5.70	11.89	10.61	16.40	16.31
A–5	2.79	3.77	12.85	10.28	15.64	14.05
A–6	3.57	5.40	12.33	10.67	15.90	16.07
A–7	5.00	4.90	11.48	9.94	16.48	14.84
A–8	5.13	.....	12.74	.....	17.87	.....
A–9	4.23	4.97	12.71	10.53	16.94	15.50
A–10	3.44	5.02	12.74	10.57	16.18	15.59

TABLE IV

ALKALINITY OF THE ASH OF CURED AND FERMENTED TOBACCO

PLOT NO.	ALKALINITY OF ASH					
	SOLUBLE		INSOLUBLE		TOTAL	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
A–1	46.7	43.6	309.6	318.7	356.3	362.3
A–2	45.7	48.3	286.5	310.6	332.5	358.9
A–3	36.6	47.0	292.5	300.5	329.1	347.5
A–4	38.5	49.6	287.0	312.6	325.5	362.2
A–5	28.1	28.2	287.0	307.9	315.1	336.1
A–6	25.1	40.2	332.4	335.4	357.5	375.6
A–7	37.3	30.2	296.0	293.2	333.3	323.4
A–8*	30.1	.....	300.0	.....	330.1	.....
A–9	35.4	41.9	309.1	305.9	344.5	347.8
A–10	31.3	37.5	332.3	317.0	363.6	354.5

* The sample of fermented material from this plot was not received.

The nitrogen-carbon ratio of the cured and fermented samples then was ascertained (table V) and the results of smoking the experimental cigars are given in table VI.

TABLE V

NITROGEN-CARBON RATIO OF CURED AND FERMENTED TOBACCO

PLOT NO.	CARBON		NITROGEN		N–C RATIO	
	CURED	FERMENTED	CURED	FERMENTED	CURED	FERMENTED
	per cent.	*per cent.*	*per cent.*	*per cent.*		
A–1	40.25	40.74	4.42	5.17	1: 9.1	1: 7.9
A–2	42.06	40.01	4.38	5.00	1: 9.6	1: 8.2
A–3	40.96	41.16	4.29	4.81	1: 9.5	1: 8.6
A–4	42.05	41.62	4.80	4.71	1: 8.8	1: 8.8
A–5	41.86	41.17	4.47	4.95	1: 9.4	1: 8.3
A–6	40.92	41.07	4.51	4.93	1: 9.1	1: 8.3
A–7	40.95	41.56	4.57	5.14	1: 9.0	1: 8.1
A–8	42.11	.....	4.44	.....	1: 9.5	.....
A–9	40.07	41.36	4.51	5.18	1: 8.9	1: 8.0
A–10	42.24	42.03	4.24	4.45	1: 10	1: 9.4

Discussion of results

Analyses of the cured samples showed that it was not possible to materially decrease the calcium or increase the potassium content by the fertilizer treatments employed; this is shown in table II. Analyses of the fermented samples, however, as shown in table III, indicate a more narrow potassium-calcium ratio. According to table II, the sulphur content was not influenced by the large quantity of sulphate of potash additions. These results indicate that the solution of the problem may not be reached by potash applications alone. More attention must be given to those factors which have to do with the availability of soil calcium. In this connection the advisability of plowing under a crop of clover, which is a heavy calcium feeder and maintains a high nitrogen content of the soil, is questioned.

The data in table III show a marked decrease in soluble ash of the fermented, as compared with the cured samples. Probably this is due to the loss of such material in the handling of the tobacco. Considerable quantities of soil and sand may adhere to the leaves of the harvested plants, owing to a gummy covering of the leaves, and even may persist over the period of air-curing. As this gummy material disappears during fermentation, there is a chance for a noticeable loss of inorganic material in this process. At the same time, there is a possibility that an equilibrium is established between the web and the midrib or stem during fermentation, resulting in a loss of calcium from the web to the midrib or stem and an increase of substances, such as potassium, in the web at the expense of these portions.

TABLE VI

SMOKING QUALITIES OF CIGARS MADE FROM THE 1928 CROP THREE-YEAR ROTATION*

SAMPLE NO.	BURN	FIRE-HOLDING CAPACITY	COLOR OF ASH	COHERENCE OF ASH	AROMA	TASTE	TOTAL**
	points	*points*	*points*	*points*	*points*	*points*	*points*
A–1 ...	18	10	8	8	15	15	74
A–2 ...	17	10	8	6	18	15	74
A–3 ...	18	10	6	8	18	15	75
A–4 ...	19	10	8	8	18	15	78
A–5 ...	15	5	8	6	10	12	56
A–6 ...	18	10	6	8	16	14	72
A–7 ...	18	10	6	8	16	14	72
A–8 ...	18	10	8	8	18	16	78
A–9 ...	16	7	6	8	16	14	67
A–10 ...	16	10	5	8	16	14	69

* A–1 Coherent. Light gray ash. Slight char. Holds fire 6–7 minutes.
A–2 Flakes slightly. Light ash. Slight char. Holds fire 6–7 minutes.
A–3 Coherent. Medium gray ash. Slight char. Holds fire 6–7 minutes.
A–4 Coherent. Light ash. Slight char. Holds fire 6–7 minutes.
A–5 Fairly coherent. Mottled ash. Chars badly. Holds fire less than 5 minutes.
A–6 Coherent. Medium gray ash. Chars slightly. Holds fire 6–7 minutes.
A–7 Coherent. Medium gray ash. Chars slightly. Holds fire 6–7 minutes.
A–8 Coherent. Light gray ash. Chars very slightly. Holds fire 6–7 minutes.
A–9 Coherent. Medium gray ash. Chars. Holds fire 5–6 minutes.
A–10 Coherent. Dark ash. Chars. Holds fire 6–7 minutes.

** A perfect score would have been as follows:

Burn	20 points
Fire-holding capacity	10 points
Color of ash	10 points
Coherence of ash	10 points
Aroma	25 points
Taste	25 points

There has been a material increase, in most instances, in the soluble ash of the web during fermentation; these differences in percentages cannot be accounted for on the basis of a loss of organic matter during fermentation. If this is correct, then the fermentation of cigar-leaf tobacco has a significance not usually taken into consideration. In order to correctly interpret the data in tables III and IV, however, the total loss of ash constituents and organic matter should be known.

The data in table V show, with one exception, a narrowing of the nitrogen-carbon ratio in the fermentation process. They further show that there must have been a relatively small amount of nitrogen lost during the fer-

mentation process. It must be remembered, however, that the fermentation was not sufficiently prolonged to insure the best quality of tobacco. The carbon content of the cured and fermented samples is, on the whole, quite uniform.

Table VI shows that cigars made from the tobacco of plot A–5 were inferior to the others. On the whole, however, the smoking tests were unsatisfactory. We believe that the lack of sufficient fermentation and aging were of overshadowing importance. All cigars tested showed a relatively high chlorophyll content.* In order to study the effect of fertilizer treatments on tobacco by the score-card system employed, it is desirable that the tobacco be fermented and aged for a much longer period of time before being made into cigars for testing.

Conclusions

The results of these investigations appear to warrant the following conclusions:

1. The fertilizer treatments employed did not materially alter the ratio of potassium to calcium in the cured leaves.

2. Increasing the quantity of sulphate of potash in the fertilizer treatments does not result in increasing the quantity of sulphur in the leaves.

3. During the process of fermentation there is an apparent increase of soluble ash constituents in the leaves.

4. There is a narrowing of the nitrogen-carbon ratio during fermentation, but very little, if any, loss of nitrogen.

5. The effect of the fertilizer treatment on the burning qualities of the cigars made from the differently treated tobaccos, is overshadowed by insufficient fermentation and aging. Qualitative tests of these cigars showed a relatively high concentration of chlorophyll, which is not only undesirable itself, but also indicates insufficient fermentation.

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* Chlorophyll, however, may be found in many of the cigars now on the market.

A STUDY OF THE AMMONIA CONTENT OF CIGAR SMOKE[1, 2]

D. E. Haley, C. O. Jensen and Otto Olson

(WITH ONE FIGURE)

The number of cigars manufactured in the United States for the year 1913 exceeded 8,500,000,000, while in 1928 the number produced was about 7,000,000,000. These figures indicate a noticeable decrease in demand. Probably the chief cause for this is the increasing popularity of cigarettes. However, the quality of the cigars now on the market may be a factor of considerable importance. The curing, fermentation, and aging processes have a great deal to do with the development of a pleasant aroma, lack of harshness, and a mild physiological effect when smoked, but the field treatment of the tobacco itself may be of overshadowing importance.

Pennsylvania ranks high as a producer of cigar-leaf tobacco. The Pennsylvania Agricultural Experiment Station and the United States Department of Agriculture have been interested for a number of years in the factors that affect quality production. Various field treatments have been employed at the Tobacco Experiment Plots at Ephrata. Each year a considerable quantity of the tobacco produced is fermented and made into cigars for testing purposes. As a rule, the cigars are made from tobacco less than a year after the crop is harvested. Because of insufficient aging, these cigars usually produce a harsh unpleasant smoke. The burn, coherence of ash, and other qualities may, however, be studied satisfactorily.

The physiological effect and other undesirable qualities of cigar smoke have been attributed, in large measure, to the nicotine content, although cigar smoke is known to contain ammonia, aldehydes, amines, organic acids, carbon monoxide, hydrocarbons, hydrogen sulphide, hydrogen cyanide, pyridine, and many other substances. From a physiological standpoint, ammonia is an important constituent in that it may interfere with the normal action of the heart and produce other complications if present in the smoke in sufficient concentrations.

Very little has been reported on the ammonia content of cigar smoke, especially for cigars made from tobacco of known history. For this reason it was decided to make a study of this constituent in the smoke of cigars made from the experimental tobaccos.

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper no. 506.

[2] This investigation was conducted in cooperation with Dr. W. W. Garner, of the U. S. Bureau of Plant Industry, Office of Plant Nutrition and Tobacco Investigations, and Professor F. D. Gardner, Department of Agronomy of the Pennsylvania State College.

Experimental

The test cigars were made wholly of tobacco grown on 10 separate plots which received fertilizer treatments according to the plan given in a previous paper (**5**). An intermittent smoking apparatus was used. Somewhat similar methods have been employed by others. JENKINS (**6**) used an apparatus in which suction was secured by means of an aspirator which filled by a continuous inflow of water and emptied at regular intervals by means of a siphon. GARNER (**4**) retained the essential features of this apparatus, but modified it so that several cigars could be smoked simultaneously. GARNER's apparatus, however, was devised for work pertaining to the burning qualities of the cigar rather than to the chemistry of the smoke. The duration of each puff was 10 seconds; the interval between puffs was 30 seconds. WILEY (**9**) describes a similar apparatus. ASHERSON (**1**) used an aspirator which evidently was turned on and off by hand, so as to simulate the manner of smoking of the average smoker. BOGEN (**2**) states that he obtained the necessary suction by the use of a water pump which was turned on and off at regular intervals by an electric solenoid valve, operated by a contact on a Harvard kymograph. An automatic siphon arrangement was tried, but was discontinued as unsatisfactory.

Various methods have been employed for collecting the active constituents of smoke. BOGEN (**2**) reports that he collected the smoke over water, allowed it to condense for one hour and then analyzed the aqueous solution for ammonia. THOMS (**7**) employed three jars containing various quantities of 10 per cent. H_2SO_4 in order to remove the basic constituents of the smoke.

Methods of analyses employed by other workers in this field were reviewed. VICKERY and PUCHER (**8**) developed a method for estimating the ammonia in tobacco and tobacco extracts which is entirely satisfactory for work of this kind. It is based on the observation that nicotine is absorbed by permutit (a synthetic alumino-silicate) only to a very small extent, whereas ammonia may be quantitatively removed from a faintly acid solution by permutit, set free by alkali and determined by Nesslerization. The method which is fully described by VICKERY and PUCHER (**8**) and which is a modification of FOLIN and BELL'S method for the determination of ammonia in urine (**3**), was used by us and found quite satisfactory.

The smoking apparatus

Some of the apparatus previously used by us proved unsatisfactory. It was felt that an intermittent siphon could be made that would give a regular interval in the suction. An apparatus was devised which proved satisfactory (see fig. 1). It was so regulated that each puff lasted about 6.5 seconds with an interval between puffs of 35 seconds.

A is a suction flask to which continuous suction is applied by means of a laboratory vacuum pump. The amount of suction is regulated by means of a valve. *B*, *C*, and *D* are absorption tubes, each of which holds 25 cc. of 20 per cent. H_2SO_4. *E* is a glass cigar holder. *F* is a tube admitting air to the suction flask *A*, when the water level in the intermittent siphon *G* is below the level of the inverted tube *F*. When the water rises to the level of the inverted funnel, the air supply is cut off and the vacuum created in *A* draws air through the cigar. When the water reaches the top of the curved tube in *G*, it siphons out and the tube *F* is again open, air enters the suction flask *A* and no air is drawn through the cigar. *I* is a bottle holding water at a constant level, and fed from the supply bottle *J*. The rate of flow of water from *I* is regulated by means of the clamp *H*.

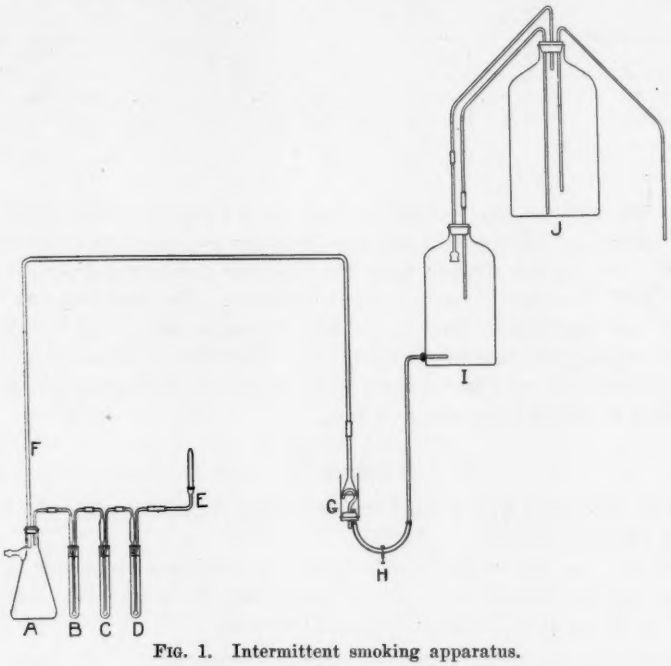

FIG. 1. Intermittent smoking apparatus.

Before smoking, the cigars were kept in a desiccator containing 43 per cent. H_2SO_4. At 25° C., according to WILSON (**10**), this should give an atmosphere having a relative humidity of 50 per cent. The ends of the cigars were cut so that all had the same circumference. Each cigar then was weighed and smoked. The small quantity of tobacco remaining at the

end of the experiment was weighed and subtracted from the original weight. In this way the weight of tobacco smoked was ascertained.

In the preliminary work it was found that 25 cc. of normal H_2SO_4 in the first tube absorbed practically all of the ammonia. Since we desired to determine nicotine, however, we used 20 per cent. H_2SO_4 instead.

Three cigars were smoked before the acid was removed. The total amount of ammonia and its relation to the total nitrogen content of the tobacco then was determined. The results are given in table I.

TABLE I

QUANTITY OF AMMONIA IN SMOKE AS RELATED TO THE TOTAL NITROGEN CONTENT OF THE ORIGINAL TOBACCO FROM WHICH THE CIGARS WERE MADE

CONSTITUENTS	SAMPLES									
	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9	A-10
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Total nitrogen	51.7	50.0	48.1	47.1	49.5	49.3	51.4	.....	51.8	44.5
Ammonia in smoke	4.7	5.4	5.5	5.6	5.4	5.4	6.0	4.8	3.5	3.6

The results show that apparently there is no relation between the ammonia content of cigar smoke and the fertilizer treatment received by the tobacco. This is not strange since the fertilizer treatment did not materially affect the nitrogen content of the tobacco. The first seven samples show a close correlation between the total nitrogen content of the tobacco and the quantity of ammonia in the smoke. Representative samples of several commercial cigars made almost wholly of well fermented tobacco showed a smoke of much lower ammonia content.

Summary

1. An apparatus was devised which proved satisfactory for the intermittent smoking of cigars.

2. There was no correlation between the fertilizer treatment of the tobacco and the ammonia content of the smoke. This may not hold true for cigars made of thoroughly fermented tobacco.

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JEAN SENEBIER
1742–1808

BRIEF PAPERS

JEAN SENEBIER

1742–1808

(WITH ONE PLATE)

> "Mais la meilleure de toutes les méthodes pour assurer la bonté de ses observations; c'est celle par laquelle on cherche à les appuyer sur de nouvelles observations faites en sens contraire: l'opposition des résultats démontre alors la justesse des premières observations."
>
> *L'Art d'Observer*, I, 209.

CALANDRINI (1743) first expressed the surmise that the leaves of plants possessed the function of collecting and absorbing dew. This induced a wealthy patrician in Geneva, CHARLES BONNET (1720–1793), to immerse shoots of a grape vine in large glass vessels. He at once observed that these shoots would be covered with innumerable air bubbles as long as sunlight lasted. After sunset, the phenomenon ceased.

This observation, which dates from 1747, opened the door to thousands of experiments at the hand of BONNET's younger contemporaries: PRIESTLEY, INGEN-HOUSZ, SENEBIER, and DE SAUSSURE. By cumulated efforts these four investigators, aided by the analytical work of LAVOISIER, succeeded in disclosing the essentials of that mystery which a later period named the phenomenon of photosynthesis, or the discovery of carbonic acid as the chief element of plant nutrition.

By association and inclination SENEBIER was predisposed to a studious life. The son of a wealthy merchant, he was born in Geneva, in 1742 and might have entered commerce, but preferred a free activity as a student of science. His family consented, except for the stipulation that the young man must take up a definite study and finish it. He chose theology and was admitted to the pastorate after three years. At an early age he had been attracted to the circle inspired and guided by BONNET, who fired his younger contemporaries by that curiosity which led to experiments rather than contemplation and philosophy.

After his graduation SENEBIER made a journey to Paris where, on BONNET's advice, he competed for a prize announced by the Haarlem academy for the best answer to the question "Wherein consists the art of making observations?" He won the prize, and returned to Switzerland where, in 1769, he became pastor at Chancy and remained in the pastorate until 1783, when he was called to Geneva as librarian of the City Library. Several works undertaken by him at this period indicate that his inclinations were divided between pure science and literary history. He translated into French the *Opuscula* of SPALLANZANI; he published a literary history of Geneva and calendared and annotated the manuscripts of the municipal library with great zeal. In 1787, he joined the staff of the

renowned *Encyclopédie méthodique* as collaborator for plant physiology. His personal work in this field was published before the Revolution, when SENEBIER returned to the little town of Rolle, carrying his laboratory equipment with him. He continued his physiological, meteorological and chemical researches there for more than ten years and printed numerous papers in the publications of several learned societies in which he held membership, notably Paris, Turin, Geneva and Lausanne. Returning to Geneva in 1799 he divided his attention between the preparation of a translation of the apocryphal books and his *Physiologie végétale* (1800) and died in 1808.

SENEBIER's "physical" experiments, historically an inspiring episode in our knowledge of photosynthesis, began as early as 1765, and their progress, which we shall try to follow, went hand in hand with theoretical and methodological contemplation of the highest order. The results of these studies constitute SENEBIER's first important book: *Essai sur l'art d'observer*, 1775. This book, I believe, is the first systematic attempt at a philosophy of the art of experimentation. The young physicist describes in great detail the mental processes, the points of view, the conscientious attention, and the skill and inventiveness necessary to an experimental worker. Each human quality fully developed, each instance of high inspiration, affords some possibility which insures good and reliable work. In its main thesis, in its appeal to the harmonic personality of the scientific worker, the book still is valid and may be read with advantage even now.

The publications of SENEBIER on plant physiology are quite numerous, but nearly all his experimental work was concentrated in the following books: 1. *Mémoires physico-chimiques sur l'influence de la lumière pour modifier les êtres des trois règnes de la nature et surtout ceux du regne végétal.* 3 vols. Geneva, 1782. 2. *Recherches sur l'influence de la lumière solaire pour métamorphoser l'air fixe en air pur par la végétation.* Geneva, 1783. 3. *Expériences sur l'action de la lumiére solaire dans la végétation.* Geneva, 1788. 4. *Physiologie végétale.* 5 vols. Geneva, 1800.

The titles of these works elucidate not only the contents, but, chronologically considered, also the historical development of SENEBIER's studies of the physical basis of photosynthesis. Scarcely any other similar series of experiments in any field of research contains a similar wealth of detail; hundreds and hundreds of times was the same arrangement repeated, now with reference to INGEN-HOUSZ, then testing the exactness of PRIESTLEY's work, then again striking a new track.

The fundamental apparatus was similar to that of INGEN-HOUSZ. The objects (leaves of plants) were deposited in a jar of water; above them and immersed in the jar, stood an inverted funnel, the neck of which was closed at its upper end and graded in order that the gas discharged by the vegetable matter might be measured. But one leaf was used in each experiment, and in each instance its surface was measured by means of a "phyllo-

meter," two glass plates, one ruled to squares, between which each leaf was measured by the number of squares which it covered. The "exhaled" gas was tested by means of VOLTA'S eudiometer.

In his first book (1779) SENEBIER denied that his plants ever developed carbonic acid under any circumstances. Green plants developed no gas in the dark. He also pointed out that oxygen was developed only by green plants in sunlight; etiolated leaves, flowers and similar structures gave only negative results. The oxygen originated in the chlorophyll-bearing parenchyma, not from leaf ribs or from epidermal structures. Even small fragments of green parenchyma secreted oxygen.

Another series of experiments was concerned with the influence of colored light on the formation of oxygen. The priority of these studies is his. He employed large, double-walled bell-bottles and tested his objects with blue (litmus solution) and yellow (curcuma extract) light. The results indicated abnormal relations, but these were considered due to the amount of light, not to its quality.

The most important of all SENEBIER'S discoveries was, however, that the presence of carbonic acid proved a deciding factor in the development of oxygen by green plants. His theory of this phenomenon was that the carbonic acid was dissociated and thus the oxygen liberated. This was proved by three important facts: a, In distilled water, leaves developed no oxygen (already pointed out by BONNET (1754); b, the amount of oxygen rose and fell with the amount of carbonic acid in the water within certain limits; c, carbonate of lime gave off no oxygen in distilled water, but if some acid was added which liberated the carbonic acid in this water, green leaves, when introduced and exposed to sunlight, would develop oxygen.

These data constitute SENEBIER'S chief contribution to the solution of the problem. He did not, however, stop at this point. He assured himself that the gas which was dissociated by the action of green leaves was the so-called "fixed gas" (carbonic acid), the amount of which, in the water, decreased considerably as the leaves acted upon it. The leaves separated the useless from the useful admixture, expelled the "pure air," *i.e.*, the oxygen, and absorbed the phlogiston as an element in their development and growth. He further concluded that the metabolism of plants consisted in the association of carbonic acid, plant juices and light, this process being confined to green tissues, probably to their resinous elements (*i.e.*, the chlorophyll). The juices of plants were replenished through the roots, which absorbed water, some solid matter, and carbonic acid (dissolved in the water).

The source of the exhaled oxygen was an important problem, and SENEBIER at once asked whether it might be attributed to atmospheric carbonic acid. His reasoning on this point is very interesting: "Carbonic acid is, and always must be, present in the lower strata of the atmosphere . . .

the problem is whether it may enter into the leaves. I confess that I do not believe carbonic acid can be carried into the leaves as a gas . . . but that it enters after having been dissolved in the water, like in charged waters."

His next conclusion was that the carbonic acid, thus present in the plant tissues, was decomposed by sunlight; the phlogiston (carbon) united with the "resinous substances" (chlorophyll), which have a great affinity for carbon.

His most important conclusion, however, was: "If the amount of oxygen given off by the leaves, is proportionate to the amount of carbonic acid in the water, and if the leaves in the water have absorbed only the carbonic acid contained therein, then the gas which is produced must be the result of a dissociation of the carbonic acid."

Scarcely less elucidating was his next conclusion, that the service of the carbonic acid lies in assisting in the formation of the acid substances contained in the plant.

Thus, in 1788, SENEBIER recognized—naturally on the basis of LAVOISIER'S analysis of carbonic acid—that "phlogiston" was identical with carbon; that carbonic acid is dissociated within the plant tissues; and finally, that the plants serve as regulators of the atmospheric content of carbonic acid.

In 1792 this subject was resumed in a paper in the *Journal de Physique.* In this paper he confirmed his former findings with the following addition: "In regard to the formation of the amount of hydrogen necessary for the production of oils and vegetable acids, this is doubtless due to the dissociation of the water, but experience has not yet taught me how it takes place in the plants." This shows that SENEBIER at this time considered water not only a medium of solution from which plants might extract certain gases dissolved therein, but as a nutritive element, subject, in itself, of an advantageous dissociation.

Substantially the same conclusions were deposited in the *Physiologie végétale,* 1800.

As is well known, INGEN-HOUSZ never recognized the fact that the secretion of oxygen depends on the presence of CO_2 in the plant. The controversy between these two investigators, at times very bitter, was continued for many years. The details, embodied in more or less tart papers, cannot now interest us deeply. To INGEN-HOUSZ belongs the credit of explaining PRIESTLEY'S discovery that living green plants "improve" the atmosphere in such a way that it will increase combustion and sustain the life of animals. This was abundantly (and independently) confirmed by SENEBIER. Neither had perfected his experiments far enough to prove conclusively that CO_2 is developed by plants in the absence of light. The share of each of these two men in the discovery of almost identical facts has been subject of much discussion and even controversy; the fact remains that INGEN-

HOUSZ admitted the development of oxygen by chlorophyll-bearing plants without the presence of CO_2. But neither *proved* the assimilation of CO_2 from the atmosphere, even though INGEN-HOUSZ observed and recognized it. Either, however, *carried Priestley's and Bonnet's observations into the field of exact experimentation*, and but few fundamental discoveries in plant physiology have been as carefully and persistently documented.

SENEBIER's studies of the cause of etiolation were quite exhaustive, but barren of valid result. More successful were his studies on the sleep-movements (afterwards continued by his friend A. DE CANDOLLE), by which it was proved that a degree of turgor remains even amidst the periodic movements.—J. CHRISTIAN BAY, *John Crerar Library, Chicago, Illinois.*

GRAFTING EXPERIMENTS WITH COTTON[1]

(WITH ONE FIGURE)

A successful method of reproducing cotton asexually offers interesting possibilities in retaining parental genotypes of this crop indefinitely. In previous papers the author has described his attempts to propagate cotton by stem cutting.[2,3] More recently some preliminary experiments in grafting cotton have been completed and it is desired to present the results of this work in the present paper. While no attempts have been made by the author to try budding in connection with the propagation of cotton, MCNAMARA and HOOTON[4] of the U. S. Cotton Breeding Station at Greenville, Texas, have succeeded in propagating cotton by budding. Grafting as a method of reproducing cotton asexually has received the attention of the writer only after poor results were secured with cuttings. Although numerous attempts have been made to root cotton stem cuttings, less than 10 per cent. of the cuttings resulted in new plants. Since little or no difficulty was experienced in securing a high percentage of callusing in stem cuttings, it was expected that grafting might be very successful.

The saddle graft method was used in these trials, fig. 1.[5] The main stem of the cotton plant to be used as the stock was trimmed to a slender wedge immediately above the lower node. Any leaves or branches below this node were removed. A scion of medium mature wood and of similar diameter to the stock was selected and cut to retain three nodes. In preparing the scion a cross-sectional cut was made immediately below a basal bud. The lower end of the scion was then split a short distance and fitted over the wedge of the stock so that the cambium layers of the scion and stock were matched

[1] Contribution from the Division of Agronomy, Texas Agricultural Experiment Station. Approved by the Director as Technical Contribution no. 130.

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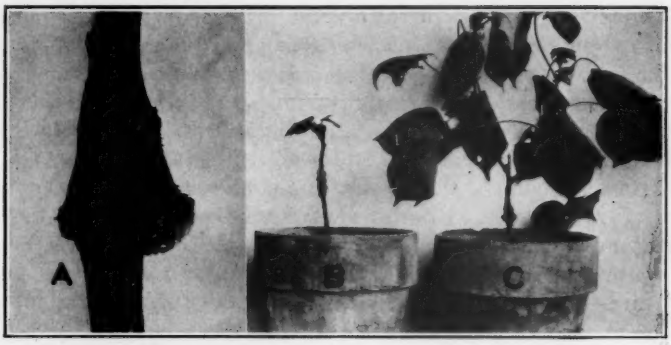

FIG. 1. Saddle grafts of cotton showing (A) enlarged view of well developed union, (B) growth of scion 15 days, and (C) 71 days after grafting.

at least on one side. After placing the scion the complete graft was wrapped with paraffined cotton string and sealed with warm paraffin. Several weeks later, after the scion had put out a leaf bud and the physiological union appeared to have taken place, the string and paraffin were removed and all leaf buds on the stock prevented from developing.

Using the saddle graft method 188 trials were made during the summer of 1930. Of this number of grafts 87, or 46.2 per cent., were successful and resulted in the production of vegetative growth and fruiting of the scion. Scions were placed at seven different dates as is shown in table I. The trials made on a specific date were comparable; however, the scions placed on the several dates were under varied conditions.

On May 14, June 25, August 9 and August 21, a total of 38, 32, 18 and 13 grafts, respectively, were made under similar conditions. The scion and stock used on these dates were all of the Lone Star variety of cotton but in each case the scion was of a different plant from the stock. The grafting was accomplished early in the morning while the temperature was moderate and the work was usually completed prior to 9 A. M. Each scion and stock was prepared and the graft completed before disturbing the next plants. Using this procedure 63.2, 75.0, 72.2 and 84.6 per cent. of the grafts attempted on May 14, June 25, August 9, and August 21, respectively, were successful. In all, 101 plants were grafted in this series and 72 scions grew, making an average of 71.3 per cent.

This was by far the most successful lot of grafts made in these preliminary trials. The plants used as stocks on May 14 were growing in 8 inch flower pots on the greenhouse bench. On September 4 at an age of 113 days the scions used on these plants were only 10 inches long but had blossoms and half mature green bolls on them. The plants used on June 25 were in 16 inch wooden tubs and had made considerably greater vegetative growth

TABLE I

SUMMARY OF PRELIMINARY GRAFTING TRIAL WITH COTTON

DATE OF TRIAL 1930	CONDITION OF TRIAL	PLANTS SUCCESSFULLY GRAFTED	PLANTS USED	SUCCESSFUL GRAFTS
May 14	Scion and stock of Lone Star, cut immediately before use. Work accomplished prior to 9 A. M.	24	38	63.2
June 25	"	24	32	75.0
August 9	"	13	18	72.2
August 21	"	11	13	84.6
Sub total	"	72	101	71.3
July 17	Scion of Willet Red Leaf and stock of Lone Star. Scion cut 48 hours prior to use and re-cut just prior to placement. Work accomplished prior to 9 A. M.	None	19	0.0
August 13	Scion and stock of Lone Star. Scion cut 1 hour prior to use. Work accomplished prior to 9 A. M.	7	29	24.1
August 20	Scion and stock of Lone Star used immediately after cutting. Work accomplished from 11 A. M. to 3 P. M.	8	39	20.5
Grand total	For all trials	87	188	46.2

than the early lot. The stems on some of these plants were 30 inches long. The development of the other lots of grafts varied all the way from the opening of a single leaf bud to three or four leaves. The information regarding the growth of the scion is offered only to show that the scion made normal development corresponding to the environment of the stock.

In the case of 19 scions of Willet Red Leaf cotton, placed on a like number as Lone Star stock on July 17, all died. These scions had been transported a considerable distance before being used and were cut from the parent plants 48 hours prior to placement on the stock. Although the scion wood was kept in loosely closed moist glass jars and a fresh cut was made on each scion as it was used, it is supposed that the delay injured the scions. On August 13 there was a delay of one hour between the time the scion-stick was cut from the parent stalk and placed on the stock. This treatment resulted in only 7 out of 29 of the scions growing, or 24.1 per cent. Again

on August 20 a total of 39 scions were placed under conditions thought to be injurious. This time the grafting was done during the heat of the day from 11 A. M. to 3 P. M. Only 8, or 20.5 per cent., of these scions grew.—*H. E.* REA, *Substation no. 5, Texas Agricultural Experiment Station, Temple, Texas.*

TEMPERATURE AS A POSSIBLE FACTOR IN REGENERATION

In view of some recent publications on the subject of regeneration the writer believes that he is justified in mentioning some observations made in 1926 while at the University of New Hampshire.

The Botany Department had two greenhouses in operation at that time, one maintained at a nearly constant temperature of 18° C. and the other at 15° C. For a time the temperature of the warm house was run above 25° C. for as much as four hours a day, but the cold house was not allowed to run above 20° C. During the course of other work the writer decided to see if temperature could have any effect on the regeneration of buds from tomato hypocotyls where the tops had been removed. Tomato was chosen because other members of the family had given striking examples of such regeneration after such operations had been performed while tomato itself refused absolutely to give any such results under normal conditions.* Accordingly pans of seedlings of tomato were prepared and started under normal conditions. When these had reached a good size, the seedlings were carefully clipped off well below the cotyledons, care being taken to see that no primordial buds such as are generally found in the axils of cotyledons remained. Checks were also kept of uninjured seedlings. Certain seedlings were then placed in the "hot" house, and an equal number in the "cold" house, each with their checks.

In a short time the writer was interested to note that practically every one of those in the "hot" house regenerated their tops by means of shoots arising from the top of the hypocotyl and thriftily produced a new set of tops. On the other hand, not one seedling of the "cold" house regenerated a new shoot or even produced a single bud. The best that they seemed able to do was to turn green and to remain alive for a time. All of these finally died. The experiment was repeated again, with the same results.

From these results the writer is inclined to believe that temperature plays a part in the regeneration of injured parts, at least in the tomato.—FRED R. CLARK, *Southeastern State Teachers' College, Durant, Oklahoma.*

* CLARK, FRED R. Bud formation on plant hypocotyls. Ann. Rept. Michigan Acad. Sci. 20: 146. 1918.

NOTES

Cleveland Meeting.—The seventh annual meeting of the American Society of Plant Physiologists at Cleveland was very largely attended, and was most successful in every way. The audience room in the Law Building of Western Reserve University was crowded most of the time during the sessions, and the papers were in the main interesting and valuable. The business sessions showed that the Society is growing substantially, and that the financial foundations are satisfactory. A spirit of optimism pervaded all of the sessions; and the banquet, at which nearly a hundred of the members were gathered, was an occasion long to be remembered. It was made the occasion of the second award of the STEPHEN HALES Prize, and the sixth award of the CHARLES REID BARNES Life Membership. With this meeting in the background, plans will no doubt go forward at once for a larger and better meeting, if possible, at New Orleans in December, 1931.

Pasadena Meeting.—The American Association for the Advancement of Science will hold its summer meeting at the California Institute of Technology, at Pasadena, in June. Tentative plans are being drawn for the participation in this meeting by the western members of the American Society of Plant Physiologists. Announcement of more details may be expected in April.

Life Membership Award.—The committee in charge of the sixth award of the CHARLES REID BARNES Life Membership in the American Society of Plant Physiologists bestowed that honor upon Dr. RODNEY HOWARD TRUE, Professor of Botany in the University of Pennsylvania, a life-long investigator of important phases of plant physiology. Dr. TRUE was born at Greenfield, Wisconsin, in 1866. His earlier training was at the University of Wisconsin, but his doctorate work was completed at Leipzig, with PFEFFER, in 1895. While at Wisconsin, he had been a student with Dr. BARNES, a circumstance which was made known by Dr. TRUE in his gracious reply to the announcement of the award. After returning from Germany, Dr. TRUE was for some years a member of the Department of Pharmacognosy at Wisconsin, and for a brief time, lecturer at Harvard University. Beginning about 1901, he was for nearly 20 years in charge of the physiological investigations of the Bureau of Plant Industry. He built up a strong organization for investigation of fundamental problems of plant life. His work on nutrition of plants, with special reference to calcium in relation to the intake of the other mineral elements, was of great signifi-

cance. In 1920 he resigned this work to become Professor of Botany and Director of the Botanic Garden, University of Pennsylvania. During a number of years he has been secretary of the Committee of One Hundred on Scientific Research, of the American Association for the Advancement of Science.

A pleasing feature of the announcement was the fact that the presentation of the award was made by Dr. R. B. HARVEY, of the University of Minnesota, who had been at one time associated with Dr. TRUE'S work in the Bureau of Plant Industry. Dr. TRUE'S response was in delightful vein, recalling his earlier connection with Dr. BARNES at Wisconsin.

Stephen Hales Prize Award.—The second award of the STEPHEN HALES Prize was made to Dr. WIGHTMAN WELLS GARNER, chemist and plant physiologist of the U. S. Department of Agriculture, who has been for many years in charge of the tobacco and plant nutrition investigations of the Bureau of Plant Industry. Dr. GARNER was born at Timmonsville, South Carolina, in 1875. A graduate of the University of South Carolina, he received his Ph.D. from Johns Hopkins University in 1900. He began his work in the Bureau of Chemistry in 1904, and became chief of the tobacco and plant nutrition work in 1909. He is known internationally for his work on photoperiodism and related phenomena.

Changes of Address.—All members of the Society should register changes of address promptly with the Secretary of the Society. PLANT PHYSIOLOGY is an expensive journal, and lost numbers break volumes, which in broken sets are worth only a fraction of their real value to the treasury. To prevent failure to deliver, notify the Secretary, and leave a forwarding address with your local postmaster. Even so, there is delay in delivery until the member sends the necessary postage for forwarding. Much trouble will be saved for everybody concerned by sending the Secretary both the old and the new address, and requesting an immediate change in your addressograph stencil.

Portraits.—Beginning with this issue, PLANT PHYSIOLOGY presents a series of portraits of famous contemporary plant physiologists. It is a great pleasure to begin this series with the portrait of Dr. LUDWIG JOST, of the Botanical Institute of Heidelberg University. In April, we hope to have a reproduction of an excellent crayon of Dr. F. F. BLACKMAN, of Cambridge University. If it can be arranged, the July and October numbers will carry portraits of Dr. F. A. WENT, of Utrecht, and Dr. N. A. MAXIMOW, of Leningrad. These are in addition to the biographical portraits which appear from time to time. In this issue we have a rare por-

trait of SENEBIER, furnished by Dr. BAY, of the John Crerar Library. Portraits of all plant physiologists appearing in PLANT PHYSIOLOGY can be obtained at 12 cents each. In complete sets, or in sets of 9 or more, the first 9 will be furnished for $1.00, postpaid. Orders should be sent to the editor, as these portraits are held at the editorial office, in Chicago.

Endowment Funds.—The American Society of Plant Physiologists has three funds to which gifts can be made at any time by those who desire to share in the permanent support of the Society's program. The program is flexible enough to permit constant adaptation of the use of funds to changing needs, so that no one need fear that funds might some day be useless. First, the STEPHEN HALES fund, which now amounts to $1100, for award of occasional prizes for outstanding contributions to the knowledge of plant physiology. Several hundred dollars should be added to this fund to guarantee income sufficient for awards at least biennially. Second, the CHARLES REID BARNES Life Membership fund, which has been allotted $1400 by action of the Executive Committee. With the provision made last year, this fund is ultimately guaranteed completion. At the same time, the way is left open for anyone who was formerly associated with BARNES, or who would consider it a privilege to share in honoring BARNES in this way, to make gifts to the fund. As life memberships are vacated, enough of these are to be added to the principal fund to bring it up to the amount required for an annual award. Third, the general endowment fund, established to care for any extraordinary service, such as publishing expensive papers, monographs, colored plates when necessary, etc. This fund has been started during the year, and contains $110. Friends of the science of plant physiology are invited to share in these projects whenever they feel like doing so. Financial strength will enable the Society to proceed with complete confidence in the future. All gifts should be sent to the Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute, Auburn, Alabama.

Back Number Wanted.—Volume I, Number 1, January, 1926, of PLANT PHYSIOLOGY is wanted by the New York State Agricultural Experiment Station, Geneva, New York. The library of the New York Station is very anxious to purchase Volume I, Number 1, of this journal to complete its file. Members and subscribers are urged to examine their files for a spare copy of this number, and communicate with Dr. JAMES G. HORSFALL, who will arrange for its purchase. The Station will appreciate greatly the cooperation in this matter of members and subscribers.

International Critical Tables.—The final volume of the first edition of the International Critical Tables was sent out by McGraw-Hill Book Co. late in 1930. This volume (VII) contains data on refractivity of gases and vapors, elements, selected solids and liquids, doubly refractive solids, organic liquids, mixtures, and electric and magnetic birefringence; kinetics of chemical processes, of biochemical reactions, and of photochemical processes. The latter section presents data on filters for Hg-vapor lamps, reaction velocities of photochemical changes, temperature coefficients, displacement of equilibria, phototropy, and quantum sensitivity. Succeeding sections consider absorption spectra of dyes, viscosity of pure liquids, free energy of chemical substances (thermodynamics), solubility of slightly soluble salts in aqueous solutions of electrolytes, optical rotatory power of solid crystals, optical rotatory power of liquids and solutions, and commercial explosives. At the close is a list of journals and books with key numbers for references. A separate index in English, French, German, and Italian covering all seven volumes, is furnished with volume VII. The publishers also announce a continuation with Annual Tables of Constants and Numerical Data, vol. VIII for 1927–28, IX for 1929, and X for 1930. Subscribers for the earlier volumes are allowed discounts for the later ones if ordered promptly.

A Textbook of Plant Physiology.—A welcome addition to the few available textbooks of plant physiology is the translation of N. A. MAXIMOW's textbook, edited by Dr. A. E. MURNEEK and Dr. R. B. HARVEY. This work is published by the McGraw-Hill Book Co., at $4.00 per copy. The book is divided into four sections: Absorption of matter and energy; Water relations of the plant; Utilization of reserve products and liberation of energy; and Growth, movement and reproduction. There are only twelve chapters in all. The careful editing has removed the evidences of translation, so that it will give the impression of an English text. It is written in clear style, is well illustrated, and should prove useful as a text. Those who prefer to begin with intake of water and salts can easily transpose the order of chapters for classes. The water relations section is valuable as a summary of MAXIMOW's theoretical conceptions and research in this field.

Enzymes.—Longmans, Green and Co. have issued a new book in their "Monographs on Biochemistry" series. It is entitled Enzymes, and is by Dr. J. B. S. HALDANE, of the Department of Biochemistry, Cambridge University. As it is based on a lecture course, it lays no claim to completeness, but has to do more with the biochemical nature of enzymes and their

actions. After an introduction, HALDANE takes up the influence of enzyme concentration and hydrogen ion concentration upon the rate of reactions. The succeeding chapters are as follows: The union of the enzyme with its substrate and related compounds; the influence of temperature and radiation on enzyme action; the course of enzyme reactions, and its mathematical theory; specificity; coenzymes, activators, kinases, and complements; the poisoning of enzymes; the purification and chemical nature of enzymes; theories of enzyme action, and classification of enzymes. Over 500 citations precede the index. The book is an interesting summary, and is more valuable than if it were crowded with a multiplicity of details from attempts to be more complete. It is based upon and extends the field cleared by BAYLISS in his earlier monograph, but without attempting to revise the earlier work. It is a valuable contribution in its field. The price of the work is $5.50, and orders should be sent to the publishers, New York.

Elements of Plant Science.—Under this title Dr. CHARLES J. CHAMBERLAIN of the University of Chicago presents an elementary textbook of Botany for beginners. The work is divided into two parts, the first part dealing with the structures and functions of seed plants, and the second with development of plants from the lowest to the highest. It is illustrated with 321 figures, and is a fresh presentation by one who has devoted his lifetime to a study of plants. A series of laboratory directions and exercises accompanies each section. Teachers of beginning botany will find much that is helpful in this book, and students will find it an attractive and stimulative introduction to plant life. It is published by McGraw-Hill Book Co., and the price, $1.90, is attractively low. Most students and investigators in the botanical fields will find it an interesting review of the fundamentals of plant life, and the organization of the plant kingdom.